ECOLOGICAL CONSEQUENCES OF RELATEDNESS: THE ROLE OF COMPETITION AND HERBIVORY IN THE COMMUNITY STRUCTURE OF CO-OCCURRING ASTERACEAE

by Michael Stastny

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Species interactions, such as competition and herbivory, are believed to be important structuring forces in plant communities. As a proxy for species similarity, the degree of relatedness among species may influence the intensity and outcomes of these interactions. In my dissertation research, I examined whether relatedness influenced the relative importance of plant competition and insect herbivory, and their feedbacks, in structuring the local community of closely related Asteraceae. Focusing on seven common, co-occurring, old-field species (genus *Solidago*, *Symphyotrichum*, and *Euthamia*), I tested the long-standing hypothesis that competition between close relatives is stronger than among more distantly related taxa. I also examined the effects of plant community structure on herbivory by a specialist outbreaking herbivore (*Trirhabda virgata*), and, in turn, the impacts of herbivory on plant competitive dynamics and community structure. Relatedness was not a reliable predictor of the intensity of competition among the focal species. Outcomes of pairwise competition between close and more distant relatives did not differ in a short-term greenhouse experiment. Over three years, community productivity and growth of individual species were comparable in field mesocosms containing closely related species or mixtures of more distantly related taxa, in spite of clear differences in competitive abilities among species. However, communities of close relatives

experienced greater invasion by other old-field species, highlighting the role of relatedness and competition in broader plant community structure. Community relatedness also determined the extent of damage by *Trirhabda*, which reflected the insect's host preference hierarchy. Specifically, the preferred genus (*Solidago*) suffered greater herbivory when growing in mixtures with the less preferred genus (*Symphyotrichum*) than when growing alone; the opposite pattern was observed for *Symphyotrichum*. In spite of differential damage, herbivory did not lead to a shift in the competitive abilities or community structure of the focal species compared to communities protected from herbivores; however, insect herbivores promoted invasion of the communities by other old-field species. In summary, my research reveals the consequences of species relatedness in trophic interactions between plants and herbivores, but not directly in plant competition, and underscores the potential feedbacks between plant community structure and herbivory over longer time scales.

BIOGRAPHICAL SKETCH

Michael (Michal) Stastny was born on the International Students' Day, November 17, 1977, in Žilina, former Czechoslovakia, to geologist parents with a great appreciation for the natural world and the outdoors. Even in his early years behind the Iron Curtain, it was evident that some sort of a career in the natural sciences was his calling, as he enthusiastically explored the forests, meadows and creeks, acquired a green thumb in his parents' and grandparents' gardens, and avidly read anything about wildlife, plants, and discovery and exploration. By his early teens, distracted by many other interests, especially the visual arts and literature, he drifted about in the rigid school system but continued to expand his knowledge of the natural systems. Shortly following the split of Czechoslovakia, his family decided to emigrate to Canada. Finding a new home in scenic Vancouver, B.C., Michael became more serious about school and focused on pursuing an academic career. In 1997, after graduating from high school and contemplating a degree in literature and philosophy, he began his undergraduate studies at Simon Fraser University (SFU), with the Dean's Scholarship in Biology. Trudging through the various prerequisites, Michael's initial, delusional aspiration to specialize in genetics quickly dissipated, and his perennial passion in ecology prevailed. Summer stints in research, working in chemical ecology of bark beetles (at SFU), and later in biocontrol of invasive plants (at CABI Bioscience, Switzerland), helped him fuel his motivation to become an ecologist. By 2001, Michael was returning to Switzerland with an independent project on the role of herbivory in plant invasion, with the mentorship of Urs Schaffner (CABI) and Elizabeth Elle (SFU). In the meantime, he was pursuing many outdoor hobbies, especially ski mountaineering, hiking, and whitewater kayaking, and immersed himself in jazz and culinary adventures. After graduating from SFU in the spring of

2002, with a Major in Biology and Minor in Physical Geography, he finished up his Swiss project (which later earned him a Young Investigator Award by the Journal of Ecology), and headed to Uppsala, Sweden, to work on willow herbivory with Stig Larsson (Swedish Agricultural University). By late summer, he moved to Padova, Italy, joining forces with Andrea Battisti (University of Padova) and other EU scientists to study geographic range expansion of a forest insect in the context of climate change. It was not easy for Michael to leave the charm of Italy, and he extended his stay and continued a series of fruitful collaborations with A. Battisti, S. Larsson, and others. Nevertheless, graduate school plans were always in the back of his mind, and by spring 2004, he was interviewing with Anurag Agrawal, at the time at University of Toronto. Through an opportune twist of fate, Michael ended up following Anurag to Bush-era America, and started his doctoral degree at the Department of Ecology and Evolutionary Biology at Cornell University in August 2004. Initially, he felt somewhat trapped in the mountainless, isolated and idealistic Ithaca, and plotted his escape through a research project in a far-away, scenic location. However, inspired by the legacy of Dick Root, Paul Feeny, and Peter Marks, and guided by Anurag, Monica Geber and Andre Kessler, Michael eventually turned his attention to local old-field plant communities to address some of the long-standing questions about the role of competition and herbivory in plant community structure. The diverse group of Cornell researchers who study plant-herbivore interactions has provided a stimulating environment for his work, and he has met many interesting people and new friends. In the meantime, he has continued his collaborative side-projects and quick travel trips in Europe, and added road cycling, pottery, film, banjo, and food and drink culture to his list of hobbies. Six years later, after a giant mesocosm experiment and countless days among the goldenrods and asters, Michael feels it's all coming together, and is looking forward to the next adventure.

Pre starkého, s vďačnostou za spomienky a dôveru

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The final years of my graduate career would have not been as fun and as fulfilling without the love and encouragement of Rebecca Wurm: thank you for your faith in me, and I look forward to our next adventure. I am also thankful to the Wurm family for their generosity, support, and interest in my work.

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and I am grateful for all her advice. A goldenrod conspirator, Andre Kessler, has been a refreshing source of tireless and creative enthusiasm in research as well as undergraduate teaching.

I would also like to thank to Dick Root, Peter Marks, and Paul Feeny, whose sage advice has been very influential in shaping my research. My past and present mentors have played a vital role in my academic career, and I want to express my gratitude to Cyril Thong, Dezene Huber, Urs Schaffner, Elizabeth Elle, Stig Larsson, and Andrea Battisti. I have also greatly benefited from the years of discussions in the Plant-Insect Interaction Group ("PIG"), and from being introduced to the R programming language and environment by Dan Rabosky and Joe Simonis. The hard-working EEB staff members have been there for me unconditionally whenever I needed their assistance.

The Agrawal and Thaler lab groups have always provided an excellent forum for ideas, helping hand, and camaraderie, and I will miss more about this gang than just the lab meetings and field station cleanups. Numerous helpers have made my dissertation possible, and I would like to thank them for their hours of sweat in the field or greenhouse, or their infinite patience in the lab. They include the past and present members of the Agrawal and Thaler labs, and, importantly, several undergraduate helpers including Michael Gilmore (who volunteered his summer days to help me build the mesocosm experiment), Nichole Becker (who helped me collect thousands of *Trirhabda* larvae, and begin my chemical endeavours into goldenrod and aster defenses), as well as Kate Hartmann, Jennifer Lamb and Margaux Birdsall.

I have been surrounded by a small but great group of friends In Ithaca, and I would like to thank Jen Koslow, Stuart Campbell, Marc Lajeunesse, Sergio Rasmann, Susan Rottschaeffer and Scott McArt for being there for me when I needed them, and for creating many fun and sometimes hazy memories. In addition, I would like to

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INTRODUCTION

Understanding the factors that shape the patterns of relative abundances and distributions of species in communities remains a central question in ecology. At larger spatial scales, environmental heterogeneity, species dispersal, and habitat filtering play a key role in creating variation in community structure (Emerson & Gillespie 2008, Cavender-Bares et al. 2009). At more fine-grain, local scales, species interactions within and between trophic levels are believed to be the key determinants of persistence and abundance of individual species in a community (Tilman 1994, Kneitel & Chase 2004). Traditionally, competition among species for resources, and herbivory or predation by higher trophic levels, have been the main, if often separate, research avenues in our efforts to explain how communities are structured and assembled (Grace & Tilman 1990, Schmitz 2008), and how species coexistence and ecological diversity are maintained (Tilman 1999). However, in spite of major conceptual and empirical advances in community ecology, the inherent complexity of natural communities has made it difficult to integrate these fields.

This relative lack of a unifying, testable framework (but see Hamback & Beckerman 2003) is particularly surprising in terrestrial plant communities, given that plant competition and plant-herbivore interactions are among the best studied areas of community ecology (Crawley 2009). Because of their sessile habit and amenability to manipulation, plants are ideal systems for studying competitive interactions among species, and their strong overlap in resource requirements and acquisition (Grace & Tilman 1990) continues to motivate research efforts to explain plant coexistence. Variation in plant community structure has also been shown to have important consequences for the diversity of other species assemblages, trophic interactions (Murdoch et al. 1972), and ecosystem function (Loreau et al. 2001, Hooper et al.

2005), including its influence on patterns and degree of herbivory (Barbosa et al. 2009). At the same time, the impressive diversity of herbivores, particularly phytophagous insects, has stimulated a large body of research on the various aspects of plant-herbivore interactions. While top-down impacts of herbivores on plant growth, fitness and abundance are well documented (Crawley 2009), fewer studies have examined how the effects of herbivory may alter plant competitive dynamics in a multi-species, community context (Brown & Gange 1992, Schadler et al. 2004, Stein et al. 2010, Scherber et al. 2010). Importantly, the potential ecological feedbacks between the effects of plant community structure on herbivory and the impacts of herbivores on plant communities, and the role of these feedbacks in shaping and maintaining local diversity, have received little attention (but see Stein et al. 2010, Scherber et al. 2010).

With the objective to integrate plant competition and herbivory in a predictive framework, my dissertation research employs a conceptual and experimental approach that focuses on the relatedness of plant species in a local community. Because of their recent shared ancestry, related species are more similar in their phenotype and ecology (phylogenetic conservatism; Harvey & Pagel 1991, Losos 2008) than more distantly related taxa. With the rapid rise in the availability of phylogenetic information in the recent years, this idea has been driving novel questions about community structure, assembly, and niche-related processes (Webb et al. 2002, Cavender-Bares et al. 2009). The majority of the studies in phyloecology have been observational, inferring ecological processes from the phylogenetic structure of communities and species traits across spatial and phylogenetic scales (Webb 2000, Cavender-Bares et al. 2004, Cavender-Bares et al. 2009). However, the concept of relatedness, even at the taxonomic level, also lends itself to promising manipulative approaches. Specifically, invoking the ecological similarity among close relatives, one can make two following

sets of experimentally testable predictions regarding the intensity of competitive interactions and patterns of attack by natural enemies within a clade of close relatives.

First, a long-standing but largely untested hypothesis predicts that, due to their significant niche overlap (MacArthur & Levins 1967, Silvertown et al. 2001), the intensity of competition for resources should be stronger among close relatives (e.g. conspecifics and species from the same genus) than among more distantly related taxa (Darwin 1859, but see Cahill et al. 2008, Valiente-Banuet & Verdu 2008). As a consequence of this intense competition, closely related species are predicted to co-occur relatively infrequently compared to more distantly related taxa (e.g. Slingsby & Verboom 2006), as one species is expected to competitively exclude the other (Harper et al. 1961). However, close relatives frequently co-occur in and may even dominate some communities, such as open successional habitats ("old fields") in eastern North America. It is possible that strong ecological interactions among close relatives could have led to niche separation (Tofts & Silvertown 2000) or character displacement in key traits (Brown & Wilson 1956, Pritchard & Schluter 2001), which have eventually weakened the intensity of negative interactions and allowed coexistence (Harper et al. 1961, Connell 1980). Yet, we may still expect that close relatives are unlikely to be more different from each other than are more distantly related taxa. This intriguing paradox raises questions about the generality of the "relatedness-competition hypothesis", and the mechanisms of local coexistence of closely related taxa.

Second, closely related plant species are also expected to share many of their natural enemies, such as herbivores and pathogens (Gilbert & Webb 2007, Pearse & Hipp 2009), due to the similarity of their defensive phenotypes (Agrawal 2007). Patterns and impacts of herbivory on a given species may thus be predicted based on the presence of close relatives growing around it. On the other hand, even closely related plant species often differ dramatically in the qualitative and quantitative levels

of their chemical and physical defense traits (Fritz & Simms 1992, Becerra & Venable 1999), producing large variation in their attractiveness and palatability to a given herbivore. In a typical plant community, herbivores are thus effectively presented with a complex mixture of more or less suitable (or unsuitable), co-occurring hosts. Most herbivores, with the exception of strict specialists, can feed on several to many plant species or even genera, but have generally evolved some degree of preference hierarchy that reflects their relative performance on the different hosts (Berenbaum 1990, Bernays & Chapman 1994). In turn, depending on the community context, a given plant species may thus benefit from associational resistance by growing among less suitable or unpalatable species belonging to a distantly related clade (e.g. through reduced probability of detection, Hamback et al. 2000), or suffer from associational susceptibility if co-occurring with its close relatives with which it shares herbivores (e.g. during spillover at high densities of herbivores, White & Whitham 2000). The local structure of a plant community may therefore create potentially important variation in the patterns and community impact of herbivory that is contingent on the relatedness of the co-occurring plant species and the preference hierarchy of the herbivore (Barbosa et al. 2009).

To examine the role of species relatedness in plant competition and herbivory, and their feedbacks, I have conducted multi-year, field manipulations of mesocosm communities, complemented with short-term experiments involving pairwise species interactions. I selected seven species of closely related, co-occurring, native Asteraceae that dominate old-field communities of central New York state (three *Solidago* spp., three *Symphyotrichum* spp., and *Euthamia graminifolia*), and examined their competitive interactions and patterns of herbivory. My dissertation builds on the long tradition of research on plant-herbivore interactions in this system that has previously focused largely on a single, dominant species (Abrahamson & Wise 1997,

Carson & Root 2000, and references therein). In contrast to most of the contemporary studies on the role of phylogenetic relationships among species in community structure (Cavender-Bares et al. 2009), my work focuses specifically on manipulative approaches that address the nature of species interactions in the context of taxonomic relatedness at fine spatial scales. Overarching the specific questions and hypotheses presented in each chapter is the central question: can we use species relatedness to evaluate the effects of plant competition, insect herbivory, and their feedbacks, on plant community structure?

In Chapter 1, I present the results of a pairwise competition experiment in a greenhouse, involving six of the seven species, that hierarchically tests the relatedness-competition hypothesis by contrasting the outcomes of competition between closely related and unrelated conspecifics, conspecifics and heterospecifics, and species from the same genus (congeners) versus from another, related genus. I revisit the relatedness-competition hypothesis in Chapter 2 with three years of data from mesocosm communities in which I manipulated species relatedness to test whether the patterns of productivity, species performance, and invasibility agree with the predicted, stronger competitive interactions in congeneric communities than in mixtures of the genera. In Chapter 3, I examine the patterns of preference and performance of a key insect herbivore, outbreaking leaf beetle *Trirhabda virgata* (Chrysomelidae), placing them in the context of insect life history traits and natural patterns of herbivory on the seven focal species. Finally, in Chapter 4, I link *Trirhabda* host preference and plant community context by exploring the feedbacks between insect herbivory and community structure, specifically by comparing whether the patterns and impacts of herbivory in the mesocosm experiment vary with community relatedness. In the Summary of my dissertation, I provide a synthetic recapitulation of the main results and their contribution to community ecology.

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CHAPTER 1

Relatedness does not predict the intensity of intraspecific or interspecific competition between plants: A greenhouse experiment with six co-occurring Asteraceae.

ABSTRACT

The degree to which species, or individuals within species, are related is likely to play an important role in their interactions, since shared ancestry should be reflected in phenotypic and ecological similarity. Similarity in resource requirements and acquisition, in turn, is expected to influence competitive interactions, one of the key ecological processes that structure communities. Yet, a long-standing prediction that close relatives compete more strongly for resources than more distantly related species or conspecific genotypes has been rarely tested experimentally. We employed a hierarchical approach in studying the effect of relatedness on intra- and inter-specific competition with six co-occurring old-field Asteraceae: three species of *Solidago* (goldenrods) and three species of *Symphyotrichum* (asters). In pairwise combinations using potted plants in a greenhouse, we manipulated relatedness of the neighbour by selecting 1) conspecific from same maternal line (half-sibling), 2) unrelated conspecific from a different maternal line (non-sibling), 3) heterospecific from the same genus (congener), or 4) heterospecific from the other genus (confamilial). Averaged across the six species, none of the measured variables – above-ground and below-ground biomass, total biomass, and root-shoot ratio – supported the prediction that the intensity of competition (measured as the outcome of the competitive effect) decreased as competing individuals became more distantly related. These results

suggest that relatedness may not be a reliable predictor of the intensity of competition in plants, at least with these six co-occurring species, and highlights the need to rigorously re-examine this long-standing paradigm.

INTRODUCTION

In recent years, ecologists have increasingly been paying attention to the potential consequences of evolutionary relationships, or relatedness in general, on ecological interactions among organisms (Losos 1994, Lovette & Hochachka 2006, Cavender-Bares et al. 2009). Because of their shared ancestry, more closely related species (or individuals of the same species) are expected to be more similar in their phenotype than more distantly related species or individuals (Darwin 1859, Harvey & Pagel 1991, Lord et al. 1995, Peterson et al. 1999). Their phenotypic similarity is then predicted to lead to similarities in many aspects of their ecology, such as environmental preferences or tolerances, life history, physiology, or resource requirements (Ackerly 2003, Cavender-Bares et al. 2004, Kraft et al. 2007). In a logical extension of the last prediction, close relatives should then face a greater overlap in their resource niches, and experience stronger competitive interactions than more distantly related species or individuals. The idea is certainly not new, having been recognized by Darwin in The Origin of Species:

"It is the most closely allied forms – varieties of the same species and species of the same and related genera – which, from having nearly the same structure, constitution and habits, generally come into the severest competition with each other…"

(Darwin 1859)

Over time, this observation became a widely accepted paradigm that has profoundly influenced our views of competitive interactions among different species as well as between heterospecifics versus between conspecifics. Often implicitly, the paradigm has shaped our evolutionary and ecological interpretation of community structure (Harper et al. 1961), from the pioneering studies of species to genus ratios by Elton (1946), to the recent focus on using species phylogenies to study community patterns such as phylogenetic overdispersion and niche overlap (Webb et al. 2002, Prinzing et al. 2008, Cavender-Bares et al. 2009). Many authors have invoked relatedness as a predictor of the strength of competition to explain patterns in community structure, or have looked for indirect evidence for this relationship by examining patterns of co-occurrence of close relatives (Diamond 1975, Sfenthourakis et al. 2005, Slingsby & Verboom 2006). However, co-occurrence (or lack thereof) is likely influenced by many additional ecological factors, including predation (Chase et al. 2002), facilitation (Valiente-Banuet & Verdu 2008), and habitat filtering (Helmus et al. 2007, Cavender-Bares et al. 2004), with competition or, ultimately, competitive exclusion, potentially playing a relatively minor role. Furthermore, even if co-occurrence patterns are generated by competitive interactions, they may represent the outcome rather than the process of competition (Grace & Tilman 1990, Goldberg 1990, Keddy 2001), the latter rather than the former being the focus of most plant competition experiments that measure the strength of competition (Connell 1983, Schoener 1983, Goldberg & Barton 1992).

While it may not be possible to quantitatively predict the magnitude of competition between species based on their position in a phylogeny (but see Valiente-Banuet & Verdu 2008), a more general qualitative prediction across a hierarchy of relatedness states that competition will be strongest among closely related than more distantly related conspecifics, and then progressively decline in intensity among

species from the same genus (congeners) and from different genera within the same family (confamilials). Eventually, descending into deeper nodes of the phylogeny (e.g. competition between species from different families), the intensity of competitive interactions may become random with respect to the species relatedness (but see Cahill et al. 2008).

The original prediction that competition is more intense between closer than between more distant relatives has been explicitly tested in very few studies. In a recent meta-analysis, Cahill et al. (2008) examined five phytometer experiments that collectively used a large number of species (142) and contrasted the competitive effect, i.e. the impact of a neighbour on the performance of the target plant (Goldberg 1990), against measures of phylogenetic relatedness using an angiosperm supertree. The authors found only weak evidence for what they termed "competition-relatedness hypothesis", with opposing relationships between phylogenetic distance and the strength of the competitive effect for eudicots and dicots. Previous, equivocal, evidence came primarily from studies using fewer species and focusing on separate slices from the spectrum of relatedness, such as competition among more versus less closely related individuals within a species (Cheplick & Kane 2004), or between congeners versus members of different genera (Resetarits 1995). To our knowledge, no study has tested the relatedness-competition hypothesis across a more complete hierarchy of relatedness that includes intra- and interspecific competition, i.e. compares the strength of competition between sibling conspecifics, non-sibling conspecifics, congeneric heterospecifics, and confamilial heterospecifics.

Here, we employ this hierarchical approach in a greenhouse competition experiment with plants grown in pots alone or with a single neighbour from the hierarchy of relatedness. We replicated the relatedness hierarchies with six herbaceous perennials from the family Asteraceae that commonly co-occur in old-field

communities of northeastern North America: three species of goldenrods (genus *Solidago*) and three species of New World asters (*Symphyotrichum*). In our analyses, we contrasted the outcomes of the competitive effect on the target plant among the four levels of relatedness on both above- and below-ground biomass, as well as relative allocation to roots and shoots, to test the general prediction that the strength of competition declines as the competing individuals are more distantly related.

METHODS

Study System

Goldenrods (*Solidago*) and New World asters (*Symphyotrichum*) are among the dominant, ubiquitous herbaceous perennials that colonize former agricultural fields, pastures, and disturbed sites in northeastern North America, persisting until the encroachment of woody species shades them out, or indefinitely with sporadic mowing. Previously likely confined to naturally disturbed or open habitats (e.g. river banks, erosional gulleys, outcrops, etc., Marks 1983), they have become widespread across the post-agricultural landscape, with 5-10 species frequently co-occurring at varying relative abundances within the same field, and even within the same microhabitats (< 1 m^2, M.S. – pers. obs.). They share many similarities in their ecology and life history, with some differences in growth form and degree of clonality between and within the two genera. At present, their phylogenetic relationships remain unresolved at the species level (Brouillet et al. 2009). Therefore, we were unable to qualitatively differentiate relatedness among the three selected species in each genus, and for our purposes treat them simply as "congeneric equals". However, in both cases they appear to belong to different subsections of their respective genera (Brouillet et al. 2009), making it highly unlikely that any two species could be sister taxa. The

species we used in our experiment were: *Solidago altissima*, *Solidago juncea*, *Solidago rugosa*, *Symphyotrichum lateriflorum*, *Symphyotrichum pilosum*, and *Symphyotrichum urophyllum*.

Competition experiment

Plant material for all six species was grown from seed collected from 12-25 maternal plants per species within a single old-field at the abandoned Whipple Farm near Ithaca, NY, USA (42°26'26" N, 76°29'49" W) in fall 2005. In early February 2006, seedlings were germinated on moist filter paper in Petri dishes and then grown in potting soil (Metro Mix, SunGro Horticulture) singly in 2" x 2" cells of planting trays for 2-4 weeks, depending on the species and germination date. On March 15-16, 2006, the seedlings were transplanted into 6" pots filled with the same potting medium, either alone (control) or with a neighbour from one of four levels of relatedness: A) same maternal line of the same species (sibling), B) different maternal line of the same species (non-sibling), C) heterospecific from the same genus (congener), or D) heterospecific from the other genus (confamilial). We excluded seedlings that were either too delayed or too advanced in their size due to differences in germination date; otherwise, there was no attempt to equalize sizes of the two seedlings in each pair and seedlings were chosen at random. Experimental treatments were replicated 10-15 times for the control and the two conspecific treatments, and 15-20 times for the congener and confamilial treatments, with near equal representation of all species combinations in the latter. Some of the replicates had to be excluded due to mortality or poor establishment. We measured the length of the longest leaf for each seedling, and counted the total number of open leaves (excluding cotyledons). We then calculated initial size of seedlings as a product of these two variables; this metric

is well correlated with the above-ground biomass of seedlings in all six species (M.S. pers. obs.).

At the onset of the competition experiment, the seedlings received a single dose of water-dissolved fertilizer (Jack's professional LX®, J.R. Peters Inc.) to stimulate growth; for the remainder of the experiments, plants were watered regularly but not fertilized. Plants were grown in a glass greenhouse at a 13L:11D photoperiod (average temperature 21°C: 9°C) for approximately 6 weeks; then on Apr 29, 2006, they were moved into a larger plastic-sheet greenhouse with a natural photoperiod and more pronounced diurnal temperate fluctuations. In both greenhouses, pots were regularly re-randomized to minimize location effects due to potential environmental gradients. By the end of the experiment, most plants were frequently experiencing drought stress on hot days, but there was only minimal shading as the pots were spaced sufficiently far apart. After the total of 10 weeks, when approximately 10% of the plants began flowering or were about to flower, we terminated the experiment (May 23-24, 2006) and separately harvested above- and below-ground biomass. In order to extract below-ground biomass, we removed soil from the root crowns by repeated washing, and took advantage of detanglers and lubricants found in hair conditioner (Suave, Unilever) in the final wash to separate entangled root crowns of the two neighbours. Above- and below-ground biomass was then dried at 60°C for 48 hours, and weighed.

Data Analysis

Once all the data had been collected, one of the two plants in each pot within the competition treatments was randomly selected as the target plant, and the other assigned as a neighbour, such that sample sizes for each pairwise combination were approximately equal. The analyses thus evaluate the effect of competition (competitive

effect, Goldberg 1990), i.e. how competition by the neighbour influences the biomass and allocation of the target plant. For all four response variables (shoot biomass, root biomass, total biomass, and root:shoot ratio), we followed two complementary analytical approaches. In the first approach, involving the data across all five treatments (i.e. including the control treatment), we tested whether the plants in the four competition treatments experienced the expected reduction in biomass or change in allocation to above- and below-ground parts. In the second approach, we focused only on the competition treatments, to hierarchically test the relatedness-competition hypothesis (see below), and to check whether the species responded differently to the experimental treatments, as might be expected.

All data were analyzed in an analysis of variance framework, using linear mixed effects models in R (R Development Core Team 2007, function lmer in package lme4), with the log-likelihood ratio test used to compare the fit of the different models and to eliminate non-significant terms (Bolker 2008). With both of the approaches described above, we started with the most complex model, fitted under maximum likelihood. This initial model included the fixed effects of treatment (five levels) and genus (two levels), their interaction, and random effects of the target species (nested within genus) and neighbour species (nested within neighbour genus). We subsequently excluded the terms involving genus and neighbour species, as none of them significantly improved the fit of the model, based on the likelihood ratio test. In other words, the response to the experimental treatments did not differ between the two genera, and the effect of the treatments on the target species was similar irrespective of the genus or species identity of the neighbour. However, using the data from the competition treatments only, the target species responded differently to the treatments, as shown when the model was fitted with a random intercept and slope at

the level of the target species (i.e. allowing the responses to the treatment to vary among the species); this term was thus included in all the models.

For the three biomass variables, the initial size of the target plant, but not the neighbour, explained a significant amount of the total variance in the model, and was included as a covariate. The total biomass of the target plant was included as a significant covariate in the analysis of root:shoot ratio, to account for the expected allometric effects of plant size on relative allocation (Keddy 2001). The response variables were transformed to improve normality and homoscedasticity; square-root transformation was used for the three biomass variables, while the natural log-transformation was used for root to shoot ratios and the initial size.

Both with the full dataset and with the dataset that included the competition treatments only, the model selection yielded the same best-fit linear model. This model, fitted under restricted maximum likelihood (Bolker 2008), included one of the two relevant covariates (above), treatment as the fixed effect, and the random effect of the target species crossed with the treatment (i.e. random intercept and slope). We defined four a priori, orthogonal contrasts for multiple comparisons, with the following specific objectives in mind, respectively: 1) Did the control (no competition) treatment differ from the competition treatments? 2) Was there a difference between the treatments involving competition between sibling conspecifics and non-sibling conspecifics? 3) Did the treatments involving intraspecific competition differ from those involving interspecific competition? And 4) was there a difference between the treatments involving competition between congeners versus between species from different genera (confamilials)? The first contrast validates whether a competitive effect occurred in our experiment; the remaining three contrasts address the predicted effects of competition along the hierarchy of decreasing relatedness. The multiple comparisons involving the defined contrasts were tested

using the general linear hypothesis testing function (glht) in the R package multcomp. For the contrasts 2, 3 and 4, we present the results from the competition treatments only.

RESULTS

The presence of a neighbour consistently and significantly suppressed above-ground, below-ground, and total biomass of the target plant compared to the control treatment (Figure 1.1, Table 1.1). On average, plants grown alone produced 47-69% more shoot biomass (z value = 7.928, $p < 0.0001$), 31%-66% more root biomass (z value = 7.817, $p < 0.0001$), and 35%-67% more total biomass (z value = 12.537, $p < 0.0001$), than the plants in the competition treatments.

Table 1.1. Summary of the linear mixed models including the control (without neighbour) treatment. For each of the four response variables of target plant growth, the overall effects of the covariates and treatment are presented. Covariates: $size_{initial}$ = initial size of target plant; $biomass_{target}$ = total biomass of target plant.

Response variable	Effect	Df	SS	F	p
Shoot biomass	$size_{initial}$	1, 221	273.46	68.70	< 0.0001
	treatment	4, 221	279.86	17.58	< 0.0001
Root biomass	$size_{initial}$	1, 219	138.71	59.00	< 0.0001
	treatment	4, 219	160.02	17.02	< 0.0001
Total biomass	$size_{initial}$	1, 218	413.20	78.03	< 0.0001
	treatment	4, 218	426.92	20.16	< 0.0001
Shoot:root ratio	$biomass_{target}$	1, 217	0.89	6.83	0.010
	treatment	4, 217	0.38	0.74	0.568

However, we found no consistent evidence that, across all six species, the extent of the competitive effect varied directionally with the degree of relatedness of the neighbour. Specifically, for neither shoot biomass, root biomass, or for total biomass was there a significant overall difference among the four competition treatments (Table 1.2). The outcomes of competition on the growth of the target plant were similar whether the neighbour was a conspecific from the same or different maternal lines (Table 1.3, contrast 1), a conspecific versus a heterospecific (Table 1.3, contrast 2), or a congener versus a confamilial (Table 1.3, contrast 3). In all cases, the initial size of the target plant explained a significant amount of variance (Table 1.1 and 1.2); the main treatment effect remained non-significant when this covariate was not included in the model.

Across all six species, root to shoot ratio did not vary significantly among the competition treatments (Table 1.2, Fig 1.2), or even between the control treatment and the competition treatments (Table 1.1, and $z = 0.203$, $p = 0.873$ for the specific contrast). The total biomass of the target plant was always a significant predictor of root to shoot ratio (Table 1.1 and 1.2). Although the six species differed significantly in their response to the competition treatments in terms of the three biomass variables, the species x treatment interaction was not significant for root to shoot ratio, indicating that all six species responded similarly to the presence and identity of the neighbours in terms of their allocation into above- and below-ground parts.

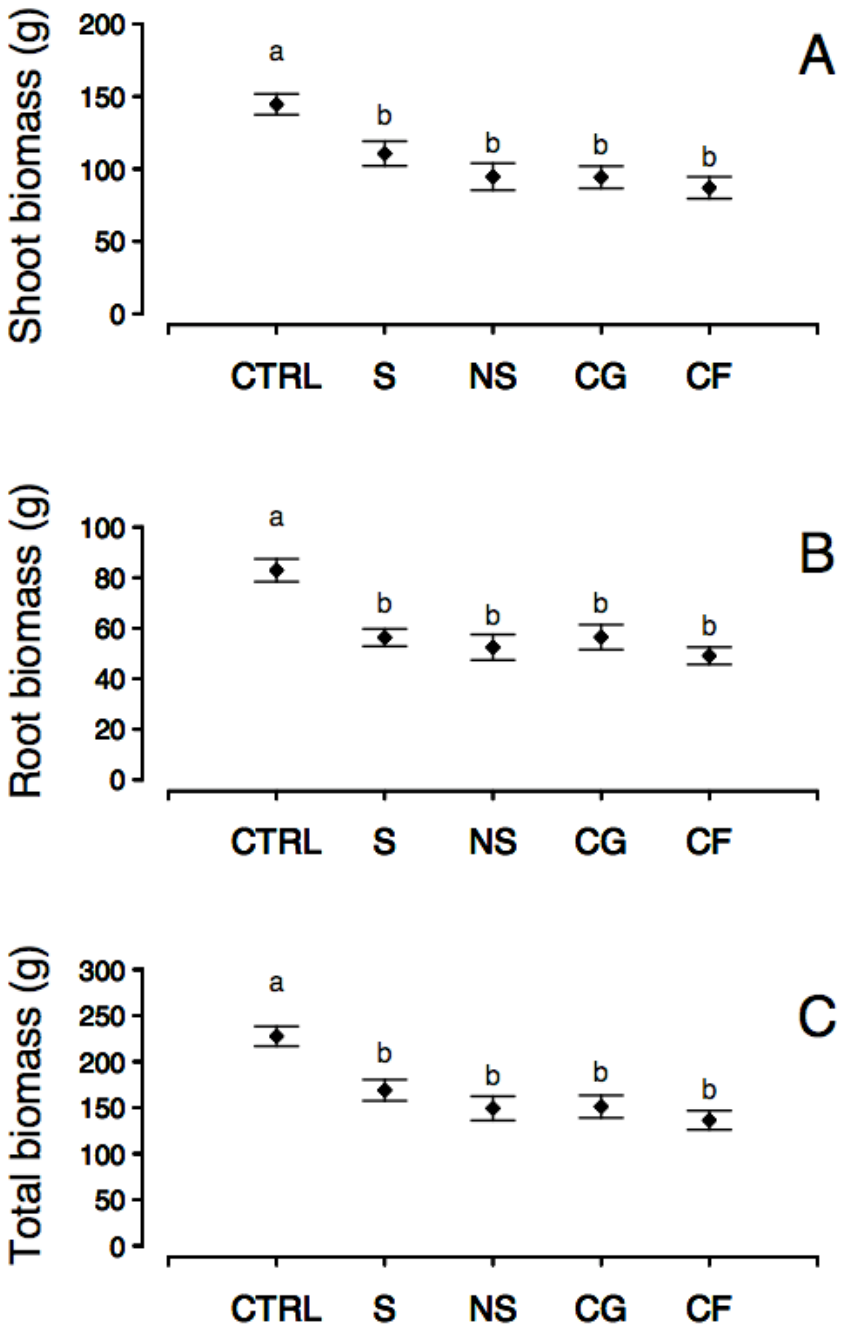

Figure 1.1. Final biomass of the target plant, averaged across six species, when grown alone (CTRL), or in competition with progressively more distantly related neighbours: sibling (S) from the same maternal family, non-sibling (NS) from a different maternal family, congener (CG), or confamilial from the other genus (CF), measured as A) shoot biomass, B) root biomass, C) total biomass. Error bars show standard error; treatments not sharing the same letter are significantly different from each other.

Table 1.2. Summary of the linear mixed models excluding the control (without neighbour) treatment. For each of the four response variables of target plant growth, the overall effects of the covariates and competition treatment are presented. Covariates: $size_{initial}$ = initial size of target plant; $biomass_{target}$ = final total biomass of target plant.

Response variable	Effect	Df	SS	F	p
Shoot biomass	$size_{initial}$	1, 167	187.67	40.69	< 0.0001
	treatment	3, 167	17.65	1.28	0.285
Root biomass	$size_{initial}$	1, 165	76.27	31.10	< 0.0001
	treatment	3, 165	2.78	0.38	0.769
Total biomass	$size_{initial}$	1, 164	265.20	44.05	< 0.0001
	treatment	3, 164	15.34	0.85	0.469
Shoot:root ratio	$biomass_{target}$	1, 163	1.74	12.22	0.001
	treatment	3, 163	0.23	0.53	0.660

Table 1.3. Summary of a priori multiple contrasts from the linear mixed models excluding the control (without neighbour) treatment, for each of the four response variables of target plant growth. Contrast 1: sibling vs. non-sibling; contrast 2: intraspecific vs. interspecific; contrast 3: congener vs. confamilial. For model specifications, see text.

Response variable	Effect	Estimate	Std. Error	z value	p
Shoot biomass	contrast 1	0.668	0.535	1.249	0.509
	contrast 2	-0.898	0.683	-1.308	0.469
	contrast 3	-0.172	0.424	-0.405	0.969
Root biomass	contrast 1	0.271	0.535	0.029	0.871
	contrast 2	0.015	0.396	0.683	1.000
	contrast 3	-0.252	0.310	-0.813	0.803
Total biomass	contrast 1	0.711	0.621	1.146	0.580
	contrast 2	-0.651	0.795	-0.818	0.797
	contrast 3	-0.269	0.488	-0.552	0.926
Shoot:root ratio	contrast 1	-0.042	0.096	-0.438	0.961
	contrast 2	0.134	0.122	1.097	0.614
	contrast 3	0.019	0.075	0.249	0.992

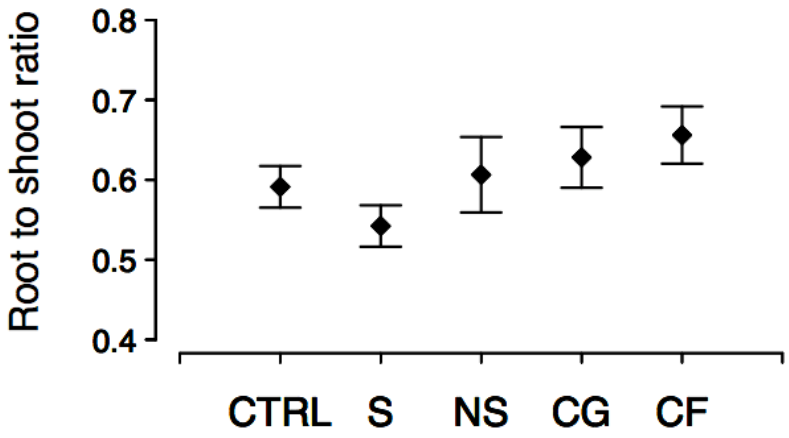

Figure 1.2. Comparison of the ratios of root to shoot biomass of the target plant, averaged across six species, when grown alone (CTRL), or in competition with progressively more distantly related neighbours: sibling (S) from the same maternal family, non-sibling (NS) from a different maternal family, congener (CG), or confamilial from the other genus (CF), measured as root to shoot ratio. Error bars show standard error.

DISCUSSION

Darwin (1859) predicted that phenotypic similarity of close relatives would lead to a stronger overlap in resource use, and, consequently, more intense resource competition than among more distant relatives. This dominant paradigm has shaped the ecological and evolutionary theory on community structure and species coexistence; yet, it has rarely been tested (but see Cahill et al. 2008).

Contrary to the expectation from the relatedness-competition paradigm, our six-species greenhouse study found little evidence that the intensity of competition was determined by the relatedness of the competitors at any level. This finding comes as a surprise, given that we employed a comprehensive hierarchy of relatedness

spanning intraspecific as well as interspecific variation by contrasting outcomes of competition between individuals from the same maternal lines (half-siblings), between individuals from different maternal lines, between congeners, and between species from two related genera from the same plant family. The presence of a neighbour consistently caused an expected reduction in both above- and below-ground plant biomass compared to a control (plant grown alone), but the magnitude of this reduction was similar among the four competition treatments. In other words, not only did more closely related versus more distantly related heterospecifics cause a similar reduction in the biomass of the target plant (competitive effect), but the magnitude of the effect in intraspecific competition did not significantly differ from that in interspecific competition (see also Vogt et al. 2010). For all variables except root to shoot ratio, the species responded differently to the competition treatments (significant species x treatment term), suggesting that in spite of species idiosyncrasies, competitive interactions among a variety of conspecifics and heterospecifics spanning a range of relatedness were functionally similar across the six species.

While the observed lack of a signal of relatedness in competition may be surprising, these results are not without a precedent in plant competition studies. For instance, Goldberg & Barton (1992) found that intraspecific competition was not usually stronger than interspecific competition, for either the competitive effect or response. On the other hand, in intraspecific competition in *Triplasis purpurea* (Poaceae), genotypes from the same maternal line experienced stronger competition than genotypes from unrelated lines (Cheplick & Kane 2004). Other studies of the effect of relatedness on intraspecific competition have generally found no evidence or only limited support for stronger competition among closer than more distant relatives (e.g. Argyres & Schmitt 1992, Monzeglio & Stoll 2008). The role of relatedness in interspecific plant competition, such as among congeners in Darwin's original

prediction, has been the focus of surprisingly few experimental studies. Cahill et al.'s (2008) meta-analysis of phytometer experiments, spanning a broad phylogenetic scale, found that the magnitude of competitive effect among species was only weakly explained by the phylogenetic relatedness of competitors, and showed a negative relationship for eudicots but not monocots. These experiments tended to be in high-nutrient or unstressed environments; in contrast, plants in our study experienced both nutrient and frequent water stress, but were not light-limited. Given that competition is expected to intensify as resources become scarce (Keddy 2001, but see Tilman 1982, Grime 1991), the similarity of competitive effects across the competition treatments in our study suggests that relatedness of competitors is relatively unimportant even under resource-limited conditions.

Changes in resource supply due to competition are predicted to alter plant allocation patterns; specifically, reduced supply of nutrients and water is expected to favour increased allocation to root biomass (Casper & Jackson 1997, Reynolds & Pacala 1993). Consequently, root to shoot ratios should increase as competition depletes below-ground resources. If competition is predicted to be stronger among close relatives, this change in allocation may be more pronounced or take place earlier. In our experiment, root to shoot ratios varied among individual species but, on average, did not differ between the controls and competition treatments, and did not increase significantly with increased relatedness of the competing plants. Given the similar effect of neighbours of differing relatedness on the biomass responses of above- and below-ground parts of the target plant, it is not surprising that the ratio of the two remained invariant across the hierarchy of relatedness. Furthermore, the lack of response in root versus shoot allocation under competition has been observed in other studies (e.g. Cahill 2003), and may be mediated by other plant responses to competition, such a changes in root morphology and interactions between above- and

below-ground parts. Therefore, the relative similarity of root to shoot ratios across the competition treatments in our study alone does not refute that relatedness may be a predictor of the intensity of competition between plants.

Competitive interactions, their effects and contingencies, and the inferences drawn from the results can be influenced by various factors, including the experimental design and environmental conditions during the study (Goldberg 1996, Gibson et al. 1999, Freckleton & Watkinson 2000). Our experiment was performed in a common environment under greenhouse conditions in the confinement of pots, and it is possible that growth, phenology, and competitive ability of the six species may differ under more natural conditions. In addition, all six species are long-lived perennials, some with strong clonal growth, and outcomes of competition after a longer time period may not reflect short-term outcomes, particularly for species with greater investment into below-ground biomass (e.g. S. juncea, S. rugosa, Abrahamson et al. 2005). On the other hand, early successional habitats, such as old fields, tend to quickly become saturated, and competition in the early stages may be critical for establishment and persistence of these species.

Interspecific competition in the present may not reflect past competitive interactions. Over time, competition could lead to competitive displacement of one of the competitors into separate habitats such that co-occurrence is rare (the "ghost of competition past", Connell 1980). Alternatively, competition could drive the evolution of traits that reduce overlap in resource use or acquisition, through character displacement (Brown & Wilson, 1956), eventually allowing species to coexist without intense competition between them (Pritchard & Schluter 2001). The first scenario is unlikely to play a role in our study; the six species we used frequently co-occur at fine spatial scales in old-field habitats of northeastern North America, with only partial segregation into microhabitats driven primarily by soil moisture gradients

(Abrahamson et al. 2005). They also likely co-occurred, perhaps to a more limited extent, in naturally disturbed or open habitats before European settlers colonized the region and old fields became widespread (Marks 1983). The second possibility, character displacement or adaptive evolution in response to competition, cannot be excluded but requires further information on divergence of traits related to resource use and/or acquisition (Silvertown et al. 2001, Ackerly 2003). However, if the interactions of the six species have intensified in the past 100-200 years as a result of increased frequency of their co-occurrence in anthropogenic successional habitats, these, and other old-field, species may in fact have had less time to diverge in their traits than species that have been restricted to shared habitats for longer periods of time. Regardless, at least in the case of interspecific competition, species traits and their relative similarity or divergence may prove to be a better predictor of the strength of competition than relatedness alone (Violle et al. 2007, but see Cadotte et al. 2009). Future studies should aim to identify key functional traits related to resource use and acquisition (e.g. water use efficiency, rooting depth, etc.) and compare their predictive ability to that of phylogenetic relationships among species. Whether such an approach could also prove to be useful in explaining variation in the strength of intraspecific competition remains to be seen, and could contribute to our understanding of the ecological consequences of genetic diversity within species.

Finally, one of the conceptual challenges of testing the role of relatedness in interspecific competition is the possibility that competitive ability may not be closely linked to the actual resource overlap between competing species. This distinction was pointed out by Aarssen (1983), who argued that resource overlap between two species may lead to two distinct evolutionary responses: selection for reduced fundamental niche overlap, or selection for reduced difference in relative competitive abilities, with either scenario preventing competitive exclusion and allowing species coexistence.

The overlooked but potentially important implications of this distinction raise questions about appropriate ways of testing Darwin's original prediction. Recent theoretical studies show that a high degree of niche overlap could, in fact, facilitate rather than preclude species coexistence (Adler et al. 2007, Abrams & Rueffler 2009). Most studies, including this one, do not attempt to explicitly quantify niche overlap between competing species, focusing instead only on competitive abilities and the process of competition. This bias may in part be due to our difficulty in identifying the relative differences in what is believed to be a very similar set of resources shared by plants (i.e. light, water, macro- and micro-nutrients), leading to more diffuse nature of competition in plant communities compared to more focused competition (e.g. for specific food plants or prey items) among animal species (Lovette & Hochachka 2006). In the future, studies should be designed to investigate both niche overlap and competitive abilities in order to provide more insight into the validity of the competition-relatedness paradigm.

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CHAPTER 2

Community relatedness and plant competition: a multi-year mesocosm experiment with old-field Asteraceae

ABSTRACT

A long-standing, yet largely untested hypothesis predicts that, due to their ecological similarity, competition among closely related species should be more intense than among more distantly related taxa. We tested this prediction in a multi-year mesocosm field experiment with seven closely related, co-occurring Asteraceae: goldenrods (*Solidago* spp.) and asters (*Symphyotrichum* spp.), and a closely related *Euthamia graminifolia*. We constructed equal-density congeneric communities (goldenrods or asters) and mixtures of the two genera, all containing *Euthamia* as a phytometer, and compared whether the mixtures performed differently than would be expected given the performance of the congeneric communities. Specifically, we employed several approaches to test whether competition was more intense when the plants were growing with their congeners than in the mixtures. First, above-ground productivity of the mixtures was comparable to that predicted by the productivity of congeneric communities, suggesting that the communities were experiencing a similar competitive environment irrespective of their relatedness. Second, while the phytometer tended to grow best in the mixtures, overall its performance in the mixtures across the three years was similar to that predicted by its biomass in the congeneric communities; furthermore, its growth was not suppressed the most when surrounded by its closer relatives (goldenrods). Third, each of the other six species reached similar biomass in its respective congeneric communities and in the mixtures,

regardless of its competitive ability or dominance; in other words, the performance of each of the species was comparable whether its competitors were close or more distant relatives. However, after three years, the mixtures were less invaded by other, mostly distantly related, old-field plant species than would be expected given the invasion of the congeneric communities. Collectively, these findings suggest that while competitive interactions play an important role in shaping the structure and diversity of local old-field communities, relatedness has only a weak impact on the intensity of competition among these close relatives.

INTRODUCTION

"It is the most closely allied forms - varieties of the same species and species of the same and related genera - which, from having nearly the same structure, constitution, and habits, generally come into the severest competition with each other..."

(Darwin 1859)

Understanding how species interact and coexist to form communities has been one of the fundamental aims of community ecology (Vellend 2010). Competition, in particular, has long been viewed as a major structuring force in communities, contributing to variation in the distribution and abundance of species and changes in community composition over time (Elton 1946, Aarssen 1983, Grace & Tilman 1990, Gurevitch 1992; but see Lamb & Cahill 2008). A number of the classic studies in community ecology have focused on the nature and consequences of competition (Tansley 1917, Gause 1934, Connell 1961), and have stimulated development of a variety of experimental approaches to quantify competition, especially among plant species (Goldberg & Barton 1992, but see also Schoener 1983). Competition has also

been invoked in the maintenance of local diversity (Connell 1980, Tilman 1982), and to explain emergent properties of communities, such as increased productivity at higher diversity levels (Tilman 1999, Loreau et al. 2001). Yet, we still have a rather incomplete understanding of the factors that determine the intensity and consequences of competitive interactions among species.

Competition has also frequently been inferred indirectly from the observed patterns of species abundance and co-occurrence (Harper et al. 1961). Specifically, closely related species are predicted to co-occur relatively infrequently compared to more distantly related species, as one species is expected to competitively exclude the other (Diamond 1975, but see Connor & Simberloff 1979). This prediction is based on a long-standing hypothesis that, due to the phenotypic and ecological similarity of taxa sharing a recent common ancestor (Harvey & Pagel 1991, Losos 2008), competition for resources should be more intense among close relatives than among more distantly related taxa (Darwin 1859). Yet, close relatives frequently co-occur in, and sometimes even dominate some communities, such as open successional habitats ("old fields") in eastern North America dominated by the Asteraceae, bogs and heaths dominated by the Ericaceae, or habitats supporting high local diversity of the Proteaceae. Furthermore, closely related species, particularly those that tend to co-occur, are not always ecologically or functionally similar (MacArthur & Levins 1967, Losos 2008 and references within). In communities dominated by close relatives, the species may possess evolutionarily conserved traits necessary for survival in that habitat, but may have diverged in other traits, including those related to resource use and acquisition (Harper et al. 1961, Cavender-Bares et al. 2004, but see Abrams 1983). At the same time, close relatives are less likely to be phenotypically more dissimilar than distantly related taxa; therefore, we still expect that competition may be more intense among co-occurring, closely related species than among co-occurring, unrelated taxa. The

juxtaposition of these predictions and observations thus presents an intriguing paradox that questions the generality and theoretical underpinnings of the prediction that competition is more intense among closely related taxa.

Over the past 150 years, the ”relatedness-competition hypothesis” became an accepted paradigm, incorporated into several foundational concepts in community ecology and evolutionary ecology (e.g. character displacement (Brown & Wilson 1956), ghost of competition past (Connell 1980), etc.), in spite of the surprising lack of experimental evidence to support it. Even in the vast body of literature on plant competition (Grace & Tilman 1990, Keddy 2001), very few studies have examined whether the intensity of competition varies with the relatedness of the competing individuals until very recently. In an observational study, Valiente-Banuet and Verdu (2007) found that interactions between woody species in the Mexican dry forest were more likely to be antagonistic (involving competition) than positive (involving facilitation) with increased relatedness. In the most synthetic test to date, Cahill et al. (2008) conducted a meta-analysis of plant competition experiments involving 142 species, including monocots and dicots. They found relatedness to be a weak and inconsistent predictor of the intensity of the competitive effect on a focal species by its surrounding plant community; the relatedness-competition relationship was not significant across all species, but showed contrasting direction when competition among eudicots versus monocots was considered. Both of these studies covered a broad phylogenetic scale, spanning many plant families; in contrast, Darwin’s original hypothesis focused on close relatives, such as congeners. To our knowledge, no single experimental study has tested the relatedness-competition hypothesis, and particularly its importance for community structure, with closely related, naturally co-occurring species.

To test the relatedness-competition paradigm in a community context, we conducted a three-year mesocosm field study involving seven closely related, naturally co-occurring, perennial Asteraceae that dominate old-field communities in central New York state, USA. Specifically, we tested whether communities composed of congeners experienced stronger competition than communities containing a mixture of the two dominant genera. We employed four complementary approaches that used the congeneric communities as a basis to predict the expected mixtures (as a sum of their congeneric parts) and compare them with the observed mixtures. First, we contrasted the total above-ground community productivity; if competition is more intense in congeneric communities, the mixtures should be more productive. Second, we utilized a competition design involving a phytometer species (belonging to a third, related genus); we predicted that its performance would be lowest when competing with species from a more closely related genus, and highest in the mixtures. Third, for each of the other six species, we compared its performance between the congeneric communities and mixtures; we predicted that each species would grow consistently worse when competing with their respective congeners. Finally, after three years, we surveyed the mesocosm communities to assess invasibility by colonizing old-field species; if the niche overlap and hence competition was reduced among more distantly related species, the resident mixtures should occupy a wider niche space and hence be more resistant to invasion.

Our study represents the first multi-year field test of the role of species relatedness in plant competitive interactions and community structure. By selecting an assemblage of co-occurring dominant species from related genera, and employing a diverse suite of measures of competitive outcomes, we provide a critical examination of Darwin's original prediction of more intense competition among close relatives.

METHODS

Study system

The study was conducted in an old field ("North Whipple") near Ithaca, central New York, USA (42°29'32.1"N, 76°25'41.2"W). Previously used for agriculture, the field was abandoned over 40 years ago (R. Root, pers. comm.), and is close to a site where a series of studies on herbivory and old-field plant community dynamics have been conducted since 1970s ("Whipple Farm", e.g. Carson & Root 2000). The field is similar to other old fields in the region, dominated by the native, perennial Asteraceae, and is mowed every 3-6 years to prevent the encroachment of woody species. The site was last mowed about 10 months prior to the beginning of the study.

We selected two locally dominant genera from the Asteraceae: *Solidago* (goldenrods) and *Symphyotrichum* (New World asters). Each genus was represented by three of the most common species that frequently co-occur even at the scale of microsites (M.S., pers. obs.): *Solidago altissima*, *So. juncea*, and *So. rugosa*, and *Symphyotrichum lateriflorum*, *Sy. pilosum*, and *Sy. urophyllum*. In our experimental design, we also included another common and ecologically similar species, more closely related to *Solidago*, *Euthamia graminifolia* (Brouillet et al. 2009), as a phytometer (see below). All seven species occur naturally at the experimental site.

Experimental setup and design

In May 2007, 120 circular holes (app. 90 cm diameter, 30 cm depth) were drilled with a mechanical auger in 40 rows in a checkerboard pattern (with 3 mesocosms per row), spaced out in an area of 200 m x 40 m to capture the natural variation in soil moisture, nutrients, and other abiotic conditions. The soil was partially removed manually, such that a strip of aluminum flashing (app. 30 cm wide and 3 m

long) could be inserted vertically along the walls of each mesocosm, extending 22-27 cm below and 3-8 cm above the soil surface. The flashing thus created a circular barrier around the perimeter of each mesocosm, preventing encroachment of other vegetation via below-ground parts, and confining the experimental communities. All soil was then returned into the mesocosms. Each mesocosm was surrounded by a wire fence (app. 2 x 2 m, 1.3 m height) to prevent herbivory by deer and rabbits. For the entire duration of the experiment, 60 of the 120 mesocosms were sprayed with a non-systemic insecticide (esfenvalerate, Ortho®, Bug-B-Gon®) once every two weeks during the peak growing season (mid May to mid October) to minimize feeding damage by insect herbivores. Esfenvalerate and a similar insecticide, fenvalerate, have been used in a number of other studies in this and other systems, and have been found to effectively reduce insect herbivory without phytotoxic effects on plants, and with minimal impacts on soil processes and fertility (Mitchell 2003, Carson & Root 2000). With the exclusion of both mammalian and insect herbivory, any changes in the structure of the experimental communities over the three years can be attributed largely to competitive interactions among the plant species, within the context of the natural variation in abiotic conditions and soil biota.

All seed material used to produce plants for this study came from old fields within 1-2 km distance from our experimental site. Seeds were collected from 20-40 individuals per species, that were growing at least 15 m apart to minimize the probability of repeated sampling from the same maternal lines. In April 2007, seeds of each species were pooled from 15-25 maternal plants per species with sufficient germination rates. The seeds were germinated in small groups directly on the moist soil surface (Metro Mix, SunGro Horticulture) in 72-cell trays in the greenhouse, at 14L:10D photoperiod. The seedlings were thinned as needed to ensure that each cell only contained a single plant. The trays were periodically reshuffled to minimize

position effects. After 5 weeks, the plants were moved into outdoor cages for several days to acclimate before being transplanted into experimental mesocosms. As much as possible, seedlings of similar size were selected for transplanting to reduce size-dependent variation in initial competitive interactions.

In late June 2006, each of the mesocosms protected from herbivory was assigned to one of three relatedness treatments, following randomized block design in four blocks. With twenty replicate mesocosms in each treatment level, we established two types of congeneric communities (goldenrod congeners or aster congeners) and a mixture of the two genera (hereafter, mixture). The congeneric communities contained three species of the respective genus, with six individual plants per species. The mixtures contained three individuals of each of the six species (i.e. three *Solidago* spp. and three *Symphyotrichum* spp). Finally, in all three types of communities, we also included three individuals of *Euthamia graminifolia*, to act as a phytometer, at the constant proportion of 1/7 of the community. Therefore, all sixty communities started at an equal initial density of 21 individuals, planted in a substitutive design, and at species richness of either four species in the congeneric communities of goldenrods or asters (*Solidago* plus phytometer, or *Symphyotrichum* plus phytometer, respectively), or seven species (equal proportion of *Solidago*, *Symphyotrichum*, and phytometer) in the mixtures.

The individual plants were planted evenly but haphazardly in each mesocosm, ensuring that the different species were intermixed within the mesocosm and avoiding concentrations of conspecifics. In the first several weeks after transplanting, mesocosms were watered periodically to aid their establishment, and lightly weeded to prevent fast-growing weeds from outgrowing the focal species. All 1260 plants established successfully and survived the first growing season.

Data collection

Between late October 2007 and early December 2007, we harvested above-ground biomass of each individual plant once most of the above-ground parts had senesced, so as not to affect the allocation of resources into below-ground parts for regrowth in the next season. All biomass was dried for a minimum of 48 hours at 60ºC, and weighed.

In the subsequent two years, the plants regrew from the below-ground parts in the spring, typically producing many additional ramets (upward of 150 total ramets per mesocosm), with *Solidago* spp. and *Euthamia* spreading clonally throughout the mesocosm. Consequently, we were no longer able to distinguish the individual plants during the 2008 and 2009 harvests, and instead pooled all ramets for each species before drying and weighing their total above-ground biomass. In each mesocosm, the species was harvested only once most of its above-ground biomass had senesced.

In July 2009, we conducted a survey of all mesocosms for other vegetation that had colonized the experimental communities during the three years. We identified and recorded all the species, and estimated their individual percent cover. In order to accurately capture the vertical complexity of the vegetation, our method allowed for counting the same area two or more times if it was occupied by two or more overlapping species; i.e. the total of percent cover values for all species can add up to more than 100%. Because of the late senescence and harvest of our focal species, we were unable to harvest and measure the above-ground biomass of the species that have invaded the mesocosms, as much of their biomass would have been lost by then.

Several of our focal species are known to segregate partially along soil moisture gradients (Abrahamson et al. 2005). In order to account for the variation in species performance due to spatial differences in soil moisture rather than due to differences in the relatedness of the community, we measured ambient soil moisture in

mesocosms using the volumetric method (FieldScout TDR 300, Spectrum Technologies). In late April 2009 and then again in early September 2009, we took three measurements in each mesocosm, avoiding the areas close to the periphery, and then averaged these values to obtain a single value for the mesocosm. Because some of the species are known to respond to seasonal fluctuations in soil moisture, rather than the absolute levels (Abrahamson et al. 2005), we included both variables as a multivariate predictor in our analyses (see below).

Statistical analysis

All of the analyses were conducted using the free software R (R Development Core Team 2007). To test our main hypothesis that the observed mixtures differed from the expected mixtures, we set a priori, orthogonal contrasts for the main effect (relatedness) specifically to compare the difference between the mixtures and the mean of the congeneric communities. A significant effect of relatedness for this contrast would indicate that mixtures performed differently than expected based on the observed performance of their congeneric parts. We also compared the two types of congeneric communities in a contrast of goldenrod and aster communities. Block was not included in any of the analyses presented here; in preliminary analyses, using mixed effects models (function lmer in R package lme4) with relatedness as a fixed effect and block as a random effect, block explained very little variance in all cases when tested using the log likelihood ratio test (Bolker 2008).

To account for temporal non-independence of the data across the three years without the issue of inflating the degrees of freedom associated with the repeated measures analysis, we used a one-way multivariate analysis of variance (MANOVA) with two covariates to analyze total community productivity (above-ground biomass) of the sixty replicate mesocosms. The model included relatedness as the main effect

and the data for the three years as a multivariate response. Soil moisture (both spring and late summer) was included as a multivariate, non-independent covariate. The dependent variable was log-transformed for the 2007 data to improve normality and homogeneity of variances.

Similar to community productivity, phytometer performance over the three years was analyzed using a one-way MANOVA, with relatedness as the main effect. We included two predictors as covariates in our models: 1) soil moisture (spring and summer) as multivariate covariate, and 2) the biomass of the rest of the community ("neighbourhood", i.e. the total biomass of the other three or six species) in each of the three years. We conducted two separate MANOVAs in order to test the specific contrasts of interest. First, we compared phytometer performance in goldenrod and aster communities, as a test of whether phytometer biomass was lower in competition with its closest relatives, the goldenrods. Second, we tested whether phytometer performance differed between any congeneric communities (goldenrods or asters) and mixtures. Soil moisture was not a significant predictor in the first MANOVA, and was dropped from the model. For both contrasts, we also tested for the effect of relatedness on productivity when the covariate of neighbourhood biomass was excluded from the model, to identify whether the phytometer responded more to the biomass rather than identity of its neighbourhood. All variables were log-transformed to improve normality and homogeneity of variances.

Individual performance of the other six species was analyzed using MANOVA in order to account for non-independence of the species within the mesocosms as well as temporal autocorrelation of mesocosms across the three years. Because of the design of our experiment, goldenrod congeneric communities do not contain any asters, and vice versa, while the mixtures contain all the species. Therefore, we split the analysis into two separate but analogous parts: one analysis compared the

performance of goldenrods in congeneric communities to those in the mixtures while ignoring the asters; the other analysis focused on the asters while ignoring the goldenrods. In both cases, above-ground biomass of each of the three species (goldenrods or asters, respectively) shared between the two community types represented a multivariate response. In order to compare mixtures (three starting individuals per species) and congeneric communities (six starting individuals per species), the dependent variable was standardized for the initial (planted) number of individuals of each species. For all species, biomass was log-transformed to meet parametric assumptions. The biomass of the phytometer, shared among all relatedness treatments, was included as a multivariate covariate, but was never a significant predictor of the species performance, and was dropped from the model.

The invasion of the mesocosms by other species was analyzed in a linear model (lm function in R), with relatedness as the main effect. The two dependent variables, species richness of invaders and total percent cover of invaders, were analyzed separately, and were log-transformed to improve normality and homogeneity of variances. Total above-ground biomass of the focal species was included as a covariate but was not significant and was dropped from the model. To test whether mixtures were less invaded than predicted based on the invasion of the congeneric communities, we set up an a priori contrast of the mean of the two congeneric communities against the mixtures.

RESULTS

Contrary to our prediction, the mixtures were not more productive than predicted by the productivity of their congeneric parts: the total above-ground biomass in mixtures was similar to the mean of the two types of congeneric communities

(Figure 2.1). This result was observed both in the overall productivity patterns across the three years (MANOVA: contrast of mixtures and mean congeneric communities: Wilks' $\lambda = 0.952$, $F_{3, 54} = 0.905$, $p = 0.445$; Table 2.1) and for each of the three years (year 1: $F_{1, 56} = 1.645$, $p = 0.205$; year 2: $F_{1, 56} = 0.164$, $p = 0.687$; year 3: $F_{1, 56} = 0.902$, $p = 0.346$). While the aster communities achieved over 65% greater biomass than the goldenrods in the first year ($F_{1, 36} = 152.0$, $p < 0.0001$), the mixtures were only 6% more productive than would be expected given the combined mean productivity of the congeneric communities.

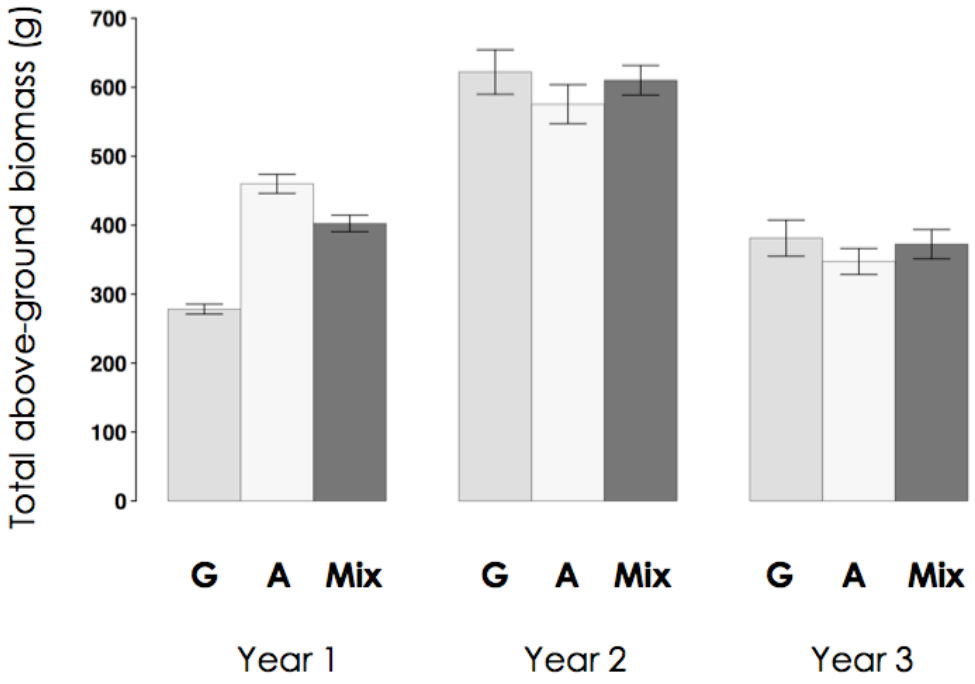

Figure 2.1. Comparison of community productivity, measured as total above-ground biomass (including the phytometer, *Euthamia graminifolia*), in the three relatedness treatments across the three years. G = four-species, goldenrod congeneric community; A = four-species, aster congeneric community; Mix = seven-species mixture of goldenrods and asters. Means ± standard errors; n = 20 per treatment.

This overyielding effect, expected both in terms of the relatedness and diversity predictions, was even smaller in the subsequent two years – only 2% and 4% overyielding (Figure 2.1) – suggesting that the congeneric communities (essentially, near monocultures at the level of genus) were not experiencing more intense competition than the mixtures. In years 2 and 3, all three types of communities reached similar above-ground biomass (Figure 2.1). Soil moisture explained a

Table 2.1. Summary of multivariate analysis of variance with community productivity (total above-ground biomass) as the three non-independent (multivariate) response variables for the three years, relatedness as the main effect (specific contrast: congeneric communities versus mixtures), and soil moisture (two variables) as a covariate. Univariate analyses for each year presented below.

MANOVA test criteria (Wilks' λ) and F statistics

Source	Wilks' λ	F	df	p
Soil moisture	0.302	14.77	2, 6	<0.0001 ***
Relatedness	0.952	0.905	1, 3	0.445

Univariate ANOVA for productivity by year:

Year	Source	df	SS	F	p
1	Soil moisture	2	0.469	4.310	0.018 *
	Relatedness	1	0.089	1.645	0.205
	Error	56	3.045		
2	Soil moisture	2	509782	36.82	<0.0001 ***
	Relatedness	1	1135	0.164	0.687
	Error	56	387682		
3	Soil moisture	2	255367	22.50	<0.0001 ***
	Relatedness	1	5121	0.902	0.346
	Error	56	317841		

significant proportion of the total variance in productivity overall and in individual years (Table 2.1); when removed as a covariate, the difference between the congeneric communities and mixtures in the first year became marginal ($F_{1, 58} = 3.092$, $p = 0.084$), suggesting that the abiotic environment was responsible for much of this effect.

The variation in the performance of the phytometer species, *Euthamia graminifolia*, did not support the prediction that competition intensity was greatest when the phytometer was competing with its closest relatives, the goldenrods (Figure 2.2). With the exception of the first year, when its above-ground biomass was nearly identical, the phytometer performance was 16% (in year 2; difference not significant) and 28% (in year 3; ANOVA: $F_{1, 38} = 4.432$, $p = 0.042$) lower in the aster than in the

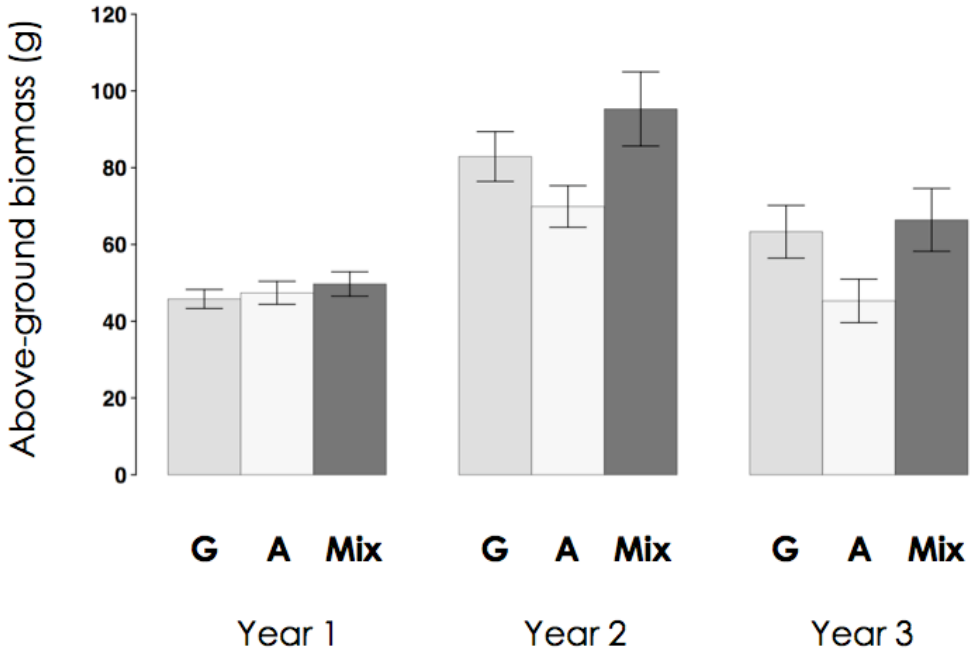

Figure 2.2. Comparison of phytometer (*Euthamia graminifolia*) performance, measured as above-ground biomass, in the three relatedness treatments across the three years. G = four-species, goldenrod congeneric community; A = four-species, aster congeneric community; Mix = seven-species mixture of goldenrods and asters. Means ± standard errors; n = 20 per treatment.

goldenrod congeneric communities (MANOVA, congeneric contrast: Wilks' λ = 0.835, $F_{1, 3}$ = 2.365; p = 0.087 overall; year 3). However, this difference disappeared when the biomass of the rest of the community was included as a covariate (MANOVA: Wilks' λ = 0.951, $F_{3, 9}$ = 0.564, p = 0.643; Table 2.2A), suggesting that the identity or relatedness of the three-species neighbourhood was less important for the performance of the phytometer than the total biomass of the neighbours.

Across the three years, phytometer biomass in the mixtures was equivalent to that predicted in the congeneric communities (Figure 2.2; MANOVA: Wilks' λ = 0.909, $F_{1, 3}$ = 1.706, p = 0.178). However, phytometer performance was 25% higher in the mixtures than would be expected given its performance in the congeneric communities in year 2 ($F_{1, 53}$ = 4.128, p = 0.047), and 22% higher in year 3 ($F_{1, 53}$ = 3.091, p = 0.084), even when accounting for neighbourhood biomass (marginal covariate overall; significant in year 2 and 3; Table 2.2B) and differences in soil moisture (significant covariate in year 3; Table 2.2B). Thus, the phytometer benefited from a more relaxed competitive environment in the mixtures compared to the congeneric communities, and this effect could not be fully explained by the biomass of its competitors or the abiotic conditions.

The individual performance of the other six species did not differ between the mixtures and their respective congeneric communities across the three years (Figure 2.3, Table 2.3; MANOVA for *Solidago* spp.: Wilks' λ = 0.788, $F_{1, 9}$ = 0.899, p = 0.539; MANOVA for *Symphyotrichum* spp.: Wilks' λ = 0.678, $F_{1, 9}$ = 1.528, p = 0.185). In other words, each species tended to reach similar per capita biomass in its congeneric community and in the mixtures, suggesting that it experienced comparable intensity of competition. These results remained consistent irrespective of the species dominance and changes in the absolute biomass of the

Table 2.2. Summary of multivariate analysis of variance with phytometer performance (above-ground biomass) as the three non-independent (multivariate) response variables for the three years, and with relatedness as the main effect. Two separate analyses presented: A) specific contrast of goldenrod versus aster congeneric communities (effect: relatedness), with the total above-ground biomass of the rest of the community as a covariate (neighbourhood) B) specific contrast of congeneric communities versus mixtures (effect: relatedness), with soil moisture (two variables) and neighbourhood as covariates. For each analysis, the univariate analyses for each year are presented below.

Table 2.2

2.2A – goldenrod versus aster

MANOVA test criteria (Wilks' λ) and F statistics

Source	Wilks' λ	F	df	p
Neighbourhood	0.658	1.679	3, 9	0.108
Relatedness	0.951	0.564	1, 3	0.642

Univariate ANOVA for productivity by year:

Year	Source	df	SS	F	p
1	Neighbourhood	3	0.043	0.184	0.906
	Relatedness	1	0.032	0.415	0.524
	Error	35	2.723		
2	Neighbourhood	3	0.396	1.015	0.398
	Relatedness	1	0.034	0.261	0.612
	Error	35	4.552		
3	Neighbourhood	3	3.124	4.377	0.010 *
	Relatedness	1	0.060	0.252	0.619
	Error	35	8.327		

Table 2.2 (Continued)

2.2B – congeneric versus mixtures

MANOVA test criteria (Wilks' λ) and F statistics

Source	Wilks' λ	F	df	p
Soil moisture	0.821	1.761	2, 6	0.114
Neighbourhood	0.735	1.862	3, 9	0.064
Relatedness	0.909	1.706	1, 3	0.178

Univariate ANOVA for productivity by year:

Year	Source	df	SS	F	p
1	Soil moisture	2	0.106	0.725	0.489
	Neighbourhood	3	0.130	0.592	0.623
	Relatedness	1	0.044	0.599	0.442
	Error	53	3.875		
2	Soil moisture	2	0.068	0.274	0.761
	Neighbourhood	3	1.413	3.815	0.015 *
	Relatedness	1	0.501	4.128	0.047 *
	Error	53	6.543		
3	Soil moisture	2	1.667	3.408	0.041 *
	Neighbourhood	3	2.767	3.771	0.016 *
	Relatedness	1	0.756	3.091	0.084
	Error	53	12.96		

species across the three years (Figure 2.3, Table 2.3); only in two species was the difference marginal (*So. altissima*, year 3: $F_{1, 9} = 3.198$, $p = 0.082$) or significant (*Sy. pilosum*, year 1: $F_{1, 9} = 5.225$, $p = 0.028$). Phytometer biomass was not a significant predictor of the performance of any of the species, and was excluded from the model as a covariate.

By the third year, mesocosm communities were invaded by a total of 48 plant species typically found in old fields in central New York, spanning a variety of plant families, and including species of native as well as exotic origin. The invaders were generally confined to the understory of the focal species, and their total biomass (not measured) was about an order of magnitude lower than that of the focal species. In agreement with the predictions, mixtures were significantly more resistant to invasion by other old-field species than was predicted from the invasion of the congeneric communities (Figure 2.4). They were invaded by 13% fewer species (t value = 2.18, $p = 0.033$) than expected given the invasion of the congeneric communities, and the total percent cover of the invaders was 24% lower than expected (t value = 2.79, $p = 0.048$).

DISCUSSION

One of the fundamental predictions in ecology is that competition among closely related species should be stronger than among more distantly related taxa (Darwin 1859, Harper et al. 1961). The results from our three-year mesocosm experiment with closely related, co-occurring old-field Asteraceae do not find much support for this paradigm. Above-ground productivity in mixtures of the two genera did not exceed the levels predicted by the productivity of the congeneric communities; phytometer performance tended to be highest in mixtures but not lowest when

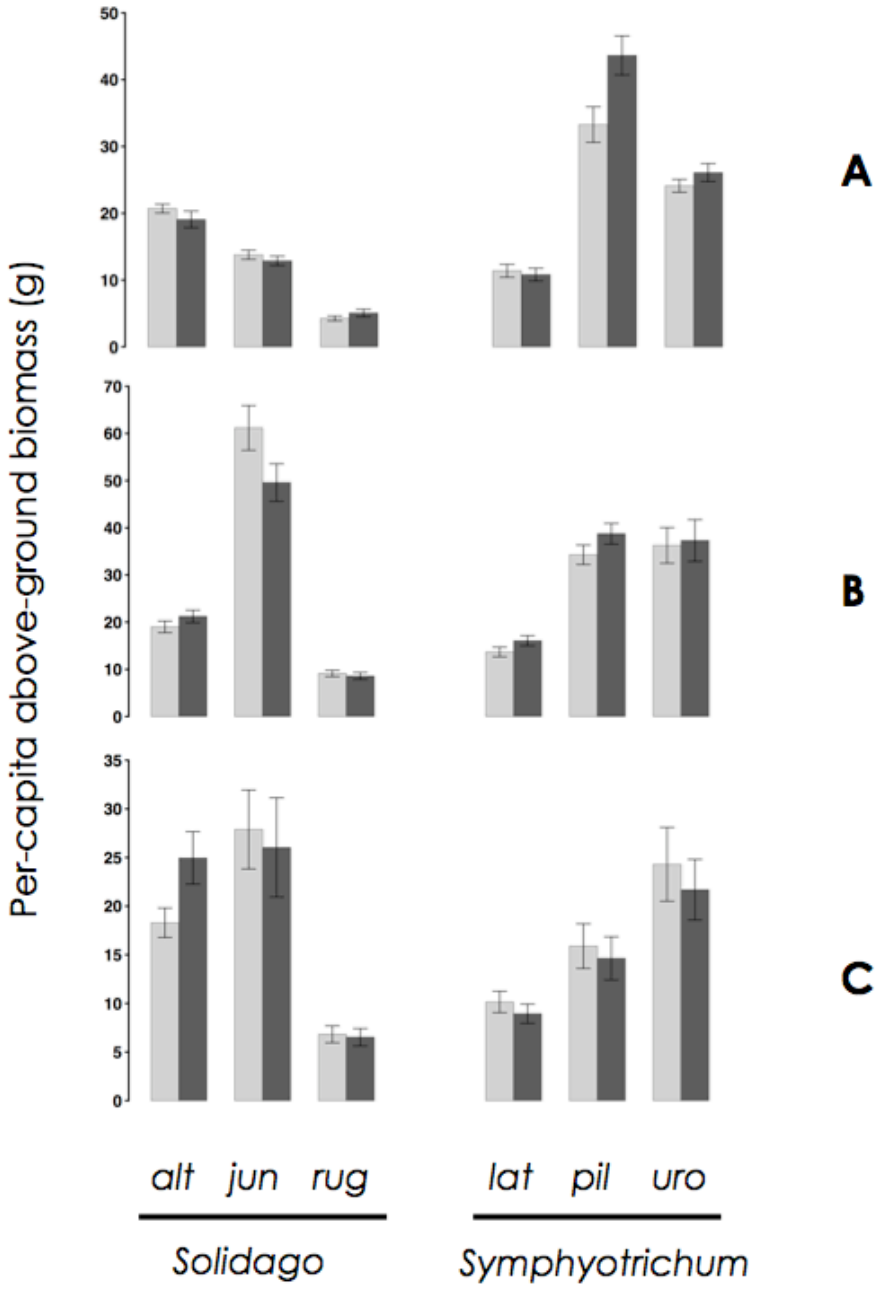

Figure 2.3. Comparison of the performance of the six species (excluding the phytometer), measured as above-ground biomass standardized per initial number of individuals, in their respective congeneric communities (light bar) and mixtures (dark bar), in A) 2007, B) 2008, C) 2009. *Solidago* species: alt = *So. altissima*; jun = *So. juncea*; rug = *So. rugosa*. *Symphyotrichum* species: lat = *Sy. lateriflorum*; pil = *Sy. pilosum*; uro = *Sy. urophyllum*. Means ± standard errors; n = 20 per treatment.

Table 2.3. Summary of multivariate analysis of variance with the performance of each species (above-ground biomass, standardized per initial number of individuals) as the non-independent (multivariate) response variables for the three years, and with relatedness as the main effect. For each species, the treatment effect (relatedness) involves a contrast of congeneric communities versus mixtures. For each species and year, the univariate analyses are presented below.

Table 2.3

MANOVA test criteria (Wilks' λ) and F statistics

Overall	Source	Wilks' λ	F	df	p
Goldenrods (*Solidago*)	Relatedness	0.212	0.899	1, 9	0.539
Asters (*Symphyotrichum*)	Relatedness	0.322	1.528	1, 9	0.185

Univariate ANOVA for species by year:

So. altissima	Source	df	SS	F	p
Year 1	Relatedness	1	0.136	2.233	0.143
	Error	38	2.306		
Year 2	Relatedness	1	0.146	1.292	0.263
	Error	38	4.301		
Year 3	Relatedness	1	0.723	3.198	0.082
	Error	38	8.587		

So. juncea	Source	df	SS	F	p
Year 1	Relatedness	1	0.053	0.894	0.350
	Error	38	2.250		
Year 2	Relatedness	1	0.411	0.068	0.796
	Error	38	229.7		
Year 3	Relatedness	1	1.178	1.007	0.322
	Error	38	44.44		

So. rugosa	Source	df	SS	F	p
Year 1	Relatedness	1	0.160	0.863	0.359
	Error	38	7.035		
Year 2	Relatedness	1	0.079	0.575	0.453
	Error	38	5.251		
Year 3	Relatedness	1	0.174	0.303	0.585
	Error	38	21.77		

Table 2.3 (Continued)

Sy. lateriflorum	Source	df	SS	F	p
Year 1	Relatedness	1	0.145	0.918	0.344
	Error	38	5.843		
Year 2	Relatedness	1	0.218	2.086	0.157
	Error	38	3.859		
Year 3	Relatedness	1	0.212	0.722	0.401
	Error	38	13.93		

Sy. pilosum	Source	df	SS	F	p
Year 1	Relatedness	1	0.684	5.225	0.028 *
	Error	38	4.847		
Year 2	Relatedness	1	0.149	1.754	0.194
	Error	38	3.133		
Year 3	Relatedness	1	0.569	0.605	0.442
	Error	38	34.82		

Sy. urophyllum	Source	df	SS	F	p
Year 1	Relatedness	1	0.038	0.845	0.364
	Error	38	1.643		
Year 2	Relatedness	1	0.011	0.021	0.886
	Error	38	19.10		
Year 3	Relatedness	1	0.025	0.024	0.879
	Error	38	39.69		

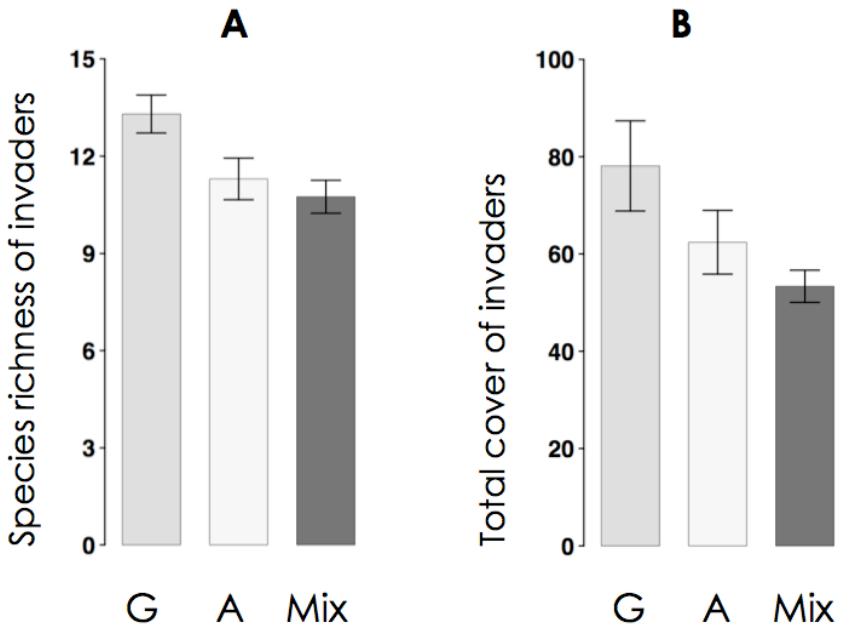

Figure 2.4. Comparison of community invasibility by year 3, between the three relatedness treatments (G = four-species, goldenrod congeneric community; A = four-species, aster congeneric community; Mix = seven-species mixture of goldenrods and asters). A) Species richness of the invaders; B) Total percent cover of the invaders, allowing overlapping cover (i.e. total in a community can be greater than 100%). Means ± standard errors; n = 20 per treatment.

growing with its closest relatives; and all species tended to grow as well in their respective congeneric communities as in mixtures. Given that the focal species clearly differed in their competitive abilities and dominance, our measures of the outcome of their competition suggest that relatedness of the species had little effect on the competitive process among these closely related old-field dominants. Nonetheless, our final measure of competitive outcomes, the degree of colonization of each mesocosm by other old-field species spanning a broad phylogenetic scale, showed that mixtures of the two genera were less invasible than predicted by the congeneric communities. These results suggest that competition did play an important role in the structure and continued assembly of old-field communities, while highlighting that relatedness may not be a strong predictor of competition among the closely related, dominant species.

Productivity

Our finding that mixtures were not more productive than what would be expected given the observed yield in the congeneric communities is surprising in the light of the numerous studies showing that productivity increases with diversity (Tilman 1999, Loreau et al. 2001, Hooper et al. 2005). In addition, this result remained invariant over the three years of the experiment, in contrast to most studies on diversity and ecosystem function, which find an increase in the diversity effect over time (Cardinale et al. 2007). Importantly, the lack of overyielding in our mixtures compared to the congeneric communities (essentially, near monocultures at the level of genus) is contrary to not just one, but two fundamental predictions: that competition should be more intense among close relatives, and that more diverse (species-rich) communities should be more productive than less diverse communities. In most diversity-productivity studies, the degree of relatedness (taxonomic or phylogenetic)

has not been considered explicitly until recently (Cadotte et al. 2009), and diversity effects have been viewed in the context of functional or trait diversity that the species represent (Tilman et al. 1997, Loreau et al. 2001). Diversity studies have typically found a steep increase in productivity between monocultures and low levels of species richness (e.g. four species), followed by an asymptote at higher diversity levels (Tilman et al. 1997, Loreau et al. 2001, Cardinale et al. 2007); the four-species congeneric communities and seven-species mixtures in our experiment fall in the diversity region where increases in productivity may be less dramatic, but are nonetheless expected to be significant (Loreau et al. 2001). More recently, post-hoc phylogenetic distance or phylodiversity-based diversity metrics have been shown to be better predictors of ecosystem functioning than the more traditional metrics, such as species or functional richness (Cadotte et al. 2008, Maherali & Klironomos 2007, Cadotte et al. 2009). Our experiment does not separate the effects of diversity and relatedness, but it consistently does not find an overyielding effect predicted both by the greater diversity and greater phylogenetic diversity (i.e. reduced relatedness) of the mixtures. As the predictions on the effects of diversity and relatedness share a similar mechanistic framework, we propose that our results can be viewed from two contrasting perspectives invoking niche complementarity (Cardinale et al. 2007).

First, we may predict that, due to their phenotypic similarity, close relatives are less likely to exhibit niche complementarity than more distantly related taxa; therefore, diversity effects on productivity may also be weaker. All seven species included in our experiment belong to the same North American clade of the tribe Asterae (family Asteraceae), and may have not had the opportunity to undergo substantial ecological and functional divergence. In other words, whether the species are in the same genus or from different genera within the same higher clade, they may all occupy a very similar niche space. Consequently, they may be experiencing strong competition for

resources, but the intensity of competition may thus be comparable in communities of close and more distant relatives, as shown in our results. The majority of diversity-productivity experiments have involved a much broader phylogenetic and functional scale than our study, selecting species from diverse plant families and functional groups (e.g. Tilman et al. 1997, Loreau et al. 2001, Cadotte et al. 2009). Presented with such a variety of plant traits, growth forms and strategies, it is easier to envision how species separate out in the available niche space, creating niche complementarity that leads to the increased productivity of more diverse communities (Cardinale et al. 2007). We have not assessed the relative phenotypic similarity of our focal species (but see Abrahamson et al. 2005); however, few studies have been able to identify key ecological traits to explain patterns in productivity or ecosystem functioning, let alone identify the ecological niches of species (Adler et al. 2007, but see Silvertown et al. 2001, Kraft et al. 2008). Furthermore, even closely related species (e.g. congeners) are believed to show greater phenotypic differences than do different genotypes of a single species; yet, overyielding and other diversity effects of comparable magnitude to those manipulating species diversity have been shown in studies manipulating genotypic diversity within a single species (e.g. Johnson et al. 2006), including *So. altissima* (Crutsinger et al. 2006). Therefore, we suggest that high ecological similarity of our focal species may be insufficient to account for the observed absence of complementarity and diversity effect.

An alternative explanation invokes ecological dissimilarity among closely related species, rather than their similarity. Old-field goldenrods and asters may have diverged over evolutionary time such that the niche overlap among congeners is, on average, comparable to that among the members of the different genera (see e.g. Abrahamson et al. 2005). This hypothesis could explain the frequent co-occurrence of congeners at the level of microsites (M.S., unpublished data): the evolution of distinct

niches due to past competitive interactions (ghost of competition past; Connell 1980) may eventually have lead to reduced competitive interactions among the species (Pritchard & Schluter 2001) and allowed their coexistence. However, this explanation only addresses the first prediction, i.e. that competition will be stronger with increased relatedness. At higher richness the species should still benefit from complementarity, especially if their niches have diverged, and should show increased productivity compared to communities with fewer species (Cardinale et al. 2007). Yet, in spite of the near doubling of species richness in our mixtures compared to the congeneric communities, productivity remained near the levels predicted by the combined productivity of the goldenrod and aster communities. This observation further reinforces the conclusion that relatedness was only a weak predictor of competitive interactions in our experimental communities.

Phytometer and individual species performance

The inclusion of the phytometer in all three types of communities provided an additional test of the link between relatedness and competition, while controlling for the productivity of the rest of the community. The phytometer, *Euthamia graminifolia*, is more closely related to goldenrods (Brouillet et al. 2009), and, importantly, is also ecologically more similar to them than to asters (M.S. unpublished data). Hence, we predicted that phytometer performance would be lowest in congeneric mesocosms of goldenrods. Instead, its performance tended to be the lowest when growing with asters (Figure 2.3), and only in the second of the three years did it reach significantly higher above-ground biomass in the mixtures than would be expected given its performance in the congeneric communities. These results agree with those found in the meta-analysis of phytometer competition experiments by Cahill et al. (2008), in which the phylogenetic relatedness between a eudicot phytometer and the surrounding

community of eudicots showed no significant relationship with the strength of the competitive effect, and relatedness was a weak overall predictor of the intensity of competition across a broader phylogeny. One important distinction is that our study captured a much narrower range of phylogenetic relatedness than the experiments analyzed by Cahill et al. (2008), and focused on co-occurring species.

During the experiment, the phytometer increased in abundance through vigorous clonal spreading, suggesting that in spite of its initial numeric disadvantage, *Euthamia* can be a strong competitor against the other species. Nonetheless, analyses of its proportional biomass (not shown) yielded similar results to those of the absolute performance: *Euthamia* performance was not lowest in the neighbourhood of its closest relatives (goldenrods), but it did tend to perform better in more species-rich mixtures than would be predicted based on its performance in the congeneric communities, at least in the last two years. We suggest that these results highlight that the effects of relatedness were less important than the effects of diversity.

The observation that mixtures did not reach greater productivity than the sum of their congeneric parts could be obscuring important differences in how the individual species or genera responded to the relatedness of the community; for instance, increased performance of one species may be countered by reduced performance of another. However, across the three years, the individual performance of the six species also varied little between the congeneric communities and mixtures, even though some species were clearly more superior competitors (Figure 2.3). In other words, whether a species was a competitive dominant or subordinate, its biomass, standardized per the initial number of individuals, was similar regardless of the relatedness of the community. This observation is particularly robust considering the among-year variation in species dominance hierarchies; despite the changes in the

relative proportional biomass of the individual species, none gained an additional competitive advantage in the mixtures.

One of the implications of these results is that, for a given species, the niche overlap in the mixtures may be comparable to that in the congeneric communities. This interpretation would suggest that the niches of the focal species are very similar, leading to competition that is intense but relatively unaffected by the degree of relatedness. The relative abundances of the species and their local coexistence may then be driven more by the differences in their competitive abilities than niche overlap per se. The distinction between niche overlap versus competitive ability was pointed out by Aarssen (1983), who hypothesized that competitive exclusion of one species by the other may be avoided either through evolutionary change leading to niche divergence, or by the selection for similarity in the competitive abilities of the species. Given the clear competitive differences among the seven species, our study does not support the idea that these closely related, co-occurring species have evolved to be equally good competitors. However, Darwin's original hypothesis pertains more directly to niche overlap among close relatives, rather than their competitive ability, and species niches have been a rather elusive concept in plant ecology (but see Silvertown et al. 2001, Adler et al. 2007). More recent theoretical studies also suggest that, rather paradoxically, high species dissimilarity may prevent stable coexistence (Abrams & Rueffler 2009).

Invasibility

An important component of community assembly is competition for unoccupied niche space in the resident community (MacDougall et al. 2009). If more distantly related taxa are characterized by a reduced niche overlap, less closely related mixtures should occupy a wider niche space and be more resistant to invasion by

colonizing species than the congeneric communities. Indeed, we found that both in terms of the number of invading species and their total abundance (percent cover), the mixtures were significantly less invaded by other old-field species after three years than would be expected given the invasibility of the congeneric communities (Figure 2.4). This result is strengthened by the fact that all three types of communities had similar total above-ground biomass of the focal species, which made up the majority of the community biomass (M. S., pers. obs.); in other words, the increased resistance of the mixtures to invasion was due to other characteristics of the competitive environment than the biomass of the resident species.

We suggest that the observed greater resistance to invasion in the mixtures may have been driven by a type of complementarity effect that reduced the open niche space in the seven-species mixtures beyond what would be expected from the sum of the unoccupied niches in the two congeneric communities. The idea that reduced availability of unoccupied niches determines the invasibility of a community is one of the classic predictions relating the niche theory and competition (Elton 1946). A number of experimental studies have shown that more diverse communities are less likely to be invaded by other species (e.g., Kennedy et al. 2002, Fargione & Tilman 2005). Unfortunately, our experimental design cannot separate the effect of relatedness from the diversity effect *per se* (i.e. species or functional richness). Because the species pool of the invaders comprised a wide variety of native and exotic species from a number of functional groups, most invaders were not closely related to our focal species. Therefore, the open niches available to invaders may be functionally comparable whether the community is a seven species mixture of the two genera (plus the phytometer), as our mixtures were, or a seven species congeneric community. In fact, the community composition of the invaders did not differ among the three community types (data not shown), showing remarkable convergence of old-field

assemblages. This observation suggests that goldenrods and asters may be ecologically quite similar in terms of supporting local diversity of other plant species.

CONCLUSION

Using four complementary approaches, we did not find support for the hypothesis that relatedness *per se* could predict the intensity of competition among the focal old-field Asteraceae. Differential competitive abilities of the species shaped the structure of mesocosm communities; yet, none of the individual species showed a consistent reduction in growth when growing with its congeners. A closer examination of the niche overlap among the species in the context of competition as well as other biotic interactions may be necessary to elucidate the mechanisms of coexistence of these foundation species of old-field communities.

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CHAPTER 3

Patterns of host use by oligophagous outbreaking beetle *Trirhabda virgata*: the role of adult performance and insect life history

ABSTRACT

The patterns of host preference in phytophagous insects are assumed to match insect performance among the hosts. However, this prediction has rarely been fully tested in insects that continue feeding as adults, even though adult host use may differ from that of the juvenile stage. In outbreaking insects, patterns of host utilization may be further complicated by the depletion of the preferred hosts, and a more polyphagous habit may be expected in the outbreaking stage. We tested this prediction and the preference-performance relationship in both larval (outbreaking) and adult stages of leaf beetle *Trirhabda virgata* (Chrysomelidae) with a series of choice and no-choice experiments with seven of its common, co-occurring host species (Asteraceae). When given a choice, the larvae displayed a strong preference hierarchy that matched their natural patterns of attack on the different hosts, and approximated relative host availability. Yet, in agreement with our prediction of increased polyphagy in the outbreaking stage, the larvae, which need to search for suitable hosts after hatching from eggs broadcast on the soil, showed relatively small differences in performance among the hosts. The more mobile adults, which continue feeding and egg laying during their lifetime, showed an even stronger preference for the same primary and most abundant host as the larvae. Importantly, this host provided higher female fecundity than any of the other hosts, suggesting that optimization of adult performance is a key factor in the more specialist patterns of adult feeding preference.

Our findings highlight the importance of life history traits and host availability in shaping the preference-performance relationship in this outbreaking insect.

INTRODUCTION

While most phytophagous insects are specialized in their host use (Bernays & Chapman 1994), many specialist insect herbivores can, and often do, utilize multiple, typically closely related, host species (Novotny & Basset 2005). On the other hand, even oligophagous species, i.e. those feeding on potentially many species or even many genera within a single plant family, tend to show a strong preference for one or few hosts (Berenbaum 1990). The general theoretical expectation is that natural selection shapes the preference hierarchy of host use reflecting the variation in insect performance on different hosts, with the host most preferred in terms of feeding and/or oviposition providing highest performance (Jaenike 1978). In reality, however, insect host preference does not always closely match performance (Thompson 1988, Mayhew 1997), and the patterns often vary between insect life stages (Scheirs 2002). Numerous factors have been invoked to explain patterns and apparent inconsistencies in host use by phytophagous insects, ranging from insect life history (e.g. Marques et al. 1994, Novotny 1995, Jervis et al. 2007) and physiology (Behmer 2009), to their interactions with competitors and natural enemies (Bernays & Graham 1988, Denno et al. 1990), to host availability (e.g. Ballabeni et al. 2003), and variation in host defense traits (Fritz & Simms 1992) and nutritional quality (Mattson 1980).

Utilization of multiple host species by some outbreaking insects (i.e. insects with irruptive population dynamics) presents additional complexity for the preference-performance theory. At outbreak densities, the insects may deplete their preferred host resource, and/or reduce its abundance for the following life stage or generation

(Barbosa & Schultz 1987). As a result, spillover of the herbivore to less preferred hosts may be a common occurrence, and natural selection may favour a more generalist life history, particularly for the outbreaking stage of the insect. Patterns of host use may then be influenced more by the relative abundances than by quality of the available host species, and the insect may exhibit reduced discrimination among the suitable hosts, or preference for the most abundant, though not necessarily optimal, hosts. In fact, a common pattern among phytophagous insects is that juvenile life stages, which invariably occur at higher densities than the adults, often utilize a wider variety of hosts than that used by the adults for feeding and/or oviposition (Bernays & Chapman 1994). In outbreaking species, a broader host range may have important implications for population dynamics by increasing the amount of available food resources and buffering against year-to-year fluctuations in host quality. Yet, relatively little attention has been paid to utilization of different hosts by outbreaking insects, perhaps because most economically important species tend to attack single host species (but see Foss & Rieske 2003, Stastny et al. 2006, Jactel & Brockerhoff 2007).

Most studies on preference-performance relationships have traditionally ignored aspects of adult performance, and focused on investigating the match, or the mismatch, between female oviposition preference and the performance (survivorship and growth) of the offspring on those hosts (see Scheirs 2002). For many insect species with non-feeding adult stages, such as many Lepidoptera, we may indeed expect a strong relationship between adult host selection and larval performance. However, many phytophagous insects, particularly in the speciose order Coleoptera, are synovigenic, i.e. they continue feeding during their adult life, commonly on the same host species, and mating and egg-laying repeatedly during that period. In insects with this type of life history, adults and larvae may differ in their dietary requirements

or detoxification mechanisms, and perform differently on the same hosts. Consequently, host choice that optimizes adult performance has been suggested to be an important and frequently overlooked component of host use in many insect species (Scheirs et al. 2000, Scheirs 2002). Because individual fitness in species with continuous oviposition will be a function of not only allocation to egg production, but also adult longevity, natural selection should favour adult feeding on hosts that maximize one or both of these aspects of lifetime fitness (Scheirs et al. 2000, Awmack & Leather 2002). Furthermore, some insects do not oviposit on their host plants and instead lay their eggs in or on the soil, on plant litter, etc.; in these species, oviposition decisions may be particularly decoupled from adult host use (e.g. Marques et al. 1994). Female host choice may therefore be only weakly correlated with larval preference and performance hierarchies, and instead reflect differences in adult performance.

The outbreaking leaf beetle *Trirhabda virgata* (Coleoptera: Chrysomelidae) is one of the most abundant and well-studied insect herbivores on dominant herbaceous perennials in old-field communities of eastern North America (Root & Cappuccino 1992, Root 1996, Uriarte 2000, Wise et al. 2006). It has typically been described and studied as a specialist on *Solidago altissima* (Asteraceae) and closely related *Solidago* (goldenrod) species (Messina 1982), but even at low population densities it can be found feeding on a number of co-occurring, related *Symphyotrichum* (New World asters) and *Euthamia* species (Blatt et al. 1999). Several life history characteristics of *Trirhabda* can be related to predictions about its host use during larval and adult stages. First, because the overwintering eggs are laid on plant litter and soil rather than on host plants, the larvae, which are the outbreaking stage of the insect, may need to utilize a broad range of available host species. Previous work on *Trirhabda*, using a phylogenetically and ecologically narrower set of host species, confirms this

prediction (Messina 1982). Second, because females continue feeding and producing eggs during their lifetime (Messina 1982), they should preferentially feed on hosts that provide the highest female performance in terms of lifetime fecundity.

We explored the patterns of preference and performance in *Trirhabda* on seven of its common old-field hosts. Using a series of choice and no-choice experiments, we tested the preference-performance relationship within and between the larval and adult stages. Given the life history of *Trirhabda*, we predicted that larvae would perform similarly on the most abundant hosts, and consequently show less discrimination among the hosts compared to the adults. We also predicted that host preference in adults would be strongly driven by differences in adult performance on different hosts.

METHODS

Study system

Trirhabda virgata (Chrysomelidae), a univoltine chewing beetle, is one of the most abundant insect herbivores on old-field perennials in the genus *Solidago* (goldenrods), especially on the dominant, clonal *So. altissima*. Larvae hatch in mid to late May from eggs laid on the soil and plant litter, and search for stems of the host plants to begin feeding on young leaves (Messina 1982). Later instars tend to feed on mature foliage, though still primarily in the upper part of the stems, leaving characteristic feeding marks. At low densities, the larvae frequently switch leaves and ramets they feed on, creating a typical pattern of low damage on most or all leaves (Kessler et al., unpublished data). However, during outbreaks, they can reach densities of up to 15-30 larvae per stem (M.S. pers. obs.) and completely defoliate plants. After 2-3 weeks of feeding, the larvae pupate in the soil. Adult beetles emerge 2-3 weeks later, usually in mid July, and begin feeding, leaving very similar feeding marks. They

continue feeding, mating, and laying eggs (upward of 250; Messina 1982) for the remainder of the summer. Their densities and the extent of feeding damage are typically several orders of magnitude lower than those of the larvae, presumably due to dispersal of adults to other sites (Herzig 1995).

The seven host species used in this study are herbaceous perennials, and locally among the most common species from the Asteraceae, the dominant plant family in old-field communities that develop on abandoned farmland and disturbed sites. In central New York state, all seven species frequently co-occur at small spatial scales within the same habitats (M.S., unpublished manuscript). The three goldenrod (*Solidago*) species, *So. altissima*, *So. juncea*, and *So. rugosa*, and the ecologically similar *Euthamia graminifolia*, spread clonally through rhizomes, and can quickly form dense patches or even near monocultures (esp. *So. altissima* and *So. rugosa*) with abundant foliage. The three *Symphyotrichum* species (New World asters; hereafter, asters), *Sy. lateriflorum*, *Sy. pilosum*, and *Sy. urophyllum*, do not have rhizomatous clonal growth, and tend to be interspersed among the goldenrods; they are common but rarely dominant, and generally have lower foliar biomass than the goldenrods. The species differ somewhat in their phenology, with the asters generally lagging behind the goldenrods in spring regrowth, but all are available as hosts both during *Trirhabda* hatching and emergence.

Larval survey

The distribution of *Trirhabda* larvae among the seven host species was surveyed at Whipple Farm, an old-field near Ithaca, central New York state, USA (42°29'32.1"N, 76°25'41.2"W), where much of the previous research on *Trirhabda* herbivory has been conducted (e.g. Messina 1982, Root 1996). In June 2006, two adjacent old-fields, where all seven hosts naturally co-occur, were surveyed during the

mid period of larval development (i.e. when older larvae were still present but hatching had already finished), capturing the bulk of the local *Trirhabda* population. While the hosts differ in their relative abundances, the two fields are similar in plant community structure and had similar *Trirhabda* population density that year (M.S., pers. obs.); subsequently the data were pooled to increase the total number of surveyed ramets. Overall, the population density was representative of non-outbreak years in this area: the larvae were common but many ramets did not have any larvae.

The survey was conducted along transects where, at regular intervals, a ramet (stem or rosette) of one of the focal species was located at random, or – for the less abundant species – was systematically searched for within a set area confined by the transects. Thus, data for the more common species represent a random sample of ramets, whereas data for the less abundant species approach an entire population sample. *Trirhabda* larvae present on each ramet were counted. Feeding damage (percent leaf area removed) was also recorded, but because this period coincides with the peak occurrence of a number of other chewing insects on goldenrods and asters, it was not possible to reliably separate *Trirhabda* damage from damage by other phytophagous insects. Several other goldenrod and aster species that also occur at this site (e.g. *Symphyotrichum* novae-angliae, *Symphyotrichum* lanceolatum, *Solidago* gigantea) can apparently be used for feeding by *Trirhabda* larvae (M.S., pers. obs.) but all are present at low abundances and appear to be minor hosts.

Larval preference experiment (choice)

In June 2009, choice trials using constructed greenhouse mesocosms were employed to evaluate larval preference for the host species. Mesocosms consisted of rings of 21 plants, with three plants in each of the seven host species, planted into an approximately 50 x 50 cm rectangle of six soaked blocks of Oasis™ floral foam. This

set-up allowed us to create randomized arrays of the host species at equal densities and size, while allowing easy access to the plants for the larvae released in the center of the ring. The plants were grown from seed (a random mixture of 6-12 maternal lines per host species, collected at Whipple Farm) in trays filled with Metro Mix™ potting soil (SunGro Horticulture) for 5-7 weeks in the greenhouse, under 13L:11D photoperiod and 21°C:10C° temperature regime. By the time of the experiment the plants had developed at least 4-5 true leaves, and we were able to select plants of similar size for each mesocosm. In order to transplant them into the mesocosms, soil was washed off their roots, and the root mass was then carefully and snugly inserted into the foam. Under these conditions, plants remain fully hydrated and can actually continue to grow for 1-2 weeks (M.S., pers. obs.), making this experimental set-up a good approximation of intact plants growing in soil.

Between June 10 and 12, 2009, we ran two trials with eight mesocosms each, using a new set of plants. Because the trials did not differ, we later pooled the data to obtain 16 replicated mesocosms. In the center of each mesocosm, we placed a group of 30 mid-size (mostly 2nd instar) *Trirhabda* larvae collected in the field from *Solidago altissima*. The larvae were fed *So. altissima* leaves for 1-2 days prior to the experiment, then starved for approximately 12 hours. To confine them within the mesocosms, a barrier of petroleum jelly (Vaseline™) was applied along the outer edges of the floral foam. Preliminary trials with the same experimental set-up showed that during the initial period (<1 hr), the larvae disperse in all directions and visit all species approximately equally, sampling the plants and continuing to move until finding a host they choose to feed on (data not shown). After 12-15 hours, we checked for their presence on each plant, recording their number and feeding damage (% leaf area removed). The majority of the larvae were on the plants, and nearly all plants showed some signs of feeding, suggesting that the larvae explored the ring. The

duration of the trials precluded the larvae from running out of leaf material on their preferred hosts, but allowed sufficient feeding for a clear hierarchy of preference to develop.

Larval performance experiment (no-choice)

To assess the relative differences in host quality among the seven species, we conducted a no-choice experiment with single *Trirhabda* larvae feeding on potted plants. The plants were grown from seeds, collected from 10-16 maternal plants for each host at Whipple Farm (see above), using the same protocol as described above. After 5-6 weeks, the plants were transplanted into 6" pots and grown in the greenhouse for another 2-3 weeks until the experiment. For each host species, 17 plants, representing a random mixture of maternal families, were used in the experiment.

Mid-sized, 2nd instar *Trirhabda* larvae were collected from *So. altissima* at Whipple Farm, fed *So. altissima* leaves for 1-2 days, and then starved for approximately 12 hrs. We selected a pool of larvae for the experiment (30.0-76.0 mg initial weight: mean 52.3 mg; median 53.00), and then allocated them to the different host species treatments, making sure that each treatment received larvae of similar mean initial weight and variance.

The experiment was conducted in a growth chamber at 20°C temperature and 13L:11D photoperiod regime. One larva was transferred onto each plant; host species were randomized within the chamber. After 7 days, the larvae were collected and weighed. Replicates with missing larvae or with larvae that had just entered the soil for pupation were excluded from the analysis; these cases were approximately equally distributed among the hosts, with 9-13 as the final number of replicates per host.

Adult preference experiment (choice)

To assess adult *Trirhabda* preference among the seven host species, we conducted a choice experiment with mixtures of potted plants in cages. The plant material was obtained as described above. We set up four mesh cages (2 m x 2 m x 2 m), each with a 1 m x 1 m square area of soil and mulch into which we placed 21 plants in 6" pots, in a randomized array with three individuals per host species. The plants were of similar size although, due to morphological and phenological differences, some species had more foliar biomass than others.

We collected *Trirhabda* beetles of unknown age in a nearby old-field, at close to a 50:50 sex ratio, and released 30 beetles inside each cage by randomly dispersing them onto the plants. After one week, we surveyed the cages and counted the beetles present on the plants. We also scored adult feeding damage by counting the number of characteristic, roughly equal-sized feeding holes. We then converted this metric into consumed leaf biomass (dry weight), using species-specific area-biomass conversions obtained by weighing leaf samples from a surplus set of plants. We took care to allow sufficient feeding without depleting foliage of any of the host species.

Adult performance experiment (no-choice)

To evaluate the relative differences in performance of adult *Trirhabda* on the seven host species, we compared short-term female fecundity in a no-choice cage experiment with groups of newly emerged beetles feeding on host monocultures. All seed material was collected at Whipple Farm, as described above. In late April, 2009, seeds from 8-10 randomly chosen maternal families were sown and germinated in trays, and the seedlings were thinned and grown in the greenhouse under 21°C:10C° average temperature regime and 13L:11D photoperiod until the end of May. Ten plants from each maternal family were then transplanted into 6" pots and kept in the

greenhouse for another 2-3 weeks. Afterwards they were hardened for 3 weeks outdoors, before being moved inside the cages on July 8-9, and acclimated for another 10 days prior to the experiment. 35 mesh cages (1 x 1 x 1 m) were constructed on a tilled field, and potted plants were placed on top of the soil inside each cage, to allow access to additional moisture and nutrients. The plants were watered regularly. For the experiment, we created five replicate monocultures per host species, in separate, randomly selected cages. Each cage contained ten plants, each from a different maternal line, with their locations randomized within the monoculture patch. In the few cases when fewer than ten maternal lines were available, we supplemented the patch with randomly chosen surplus plants from other maternal lines.

On June 14-15, we collected approximately 6000 late-instar *Trirhabda* larvae from *So. altissima* in an old-field near Lansing, central New York state, that was experiencing a localized population outbreak (mean = 4.67 larvae per ramet, median = 4.0; range 0-28; mean percent defoliation app. 25%; M.S. pers. obs.). The larvae were transferred into six mesh cages (1 x 1 x 1 m), at a density of approximately 1000 per cage, and supplied ad libitum with clipped stems of *So. altissima* inserted into Oasis™ floral foam blocks to maintain their freshness. A layer of Metro Mix™ potting soil (SunGro Horticulture), approximately 15 cm deep, was laid out in each cage for pupation. The majority of the larvae pupated into the soil within several days. After about three weeks, adult *Trirhabda* beetles began emerging from the soil. They were not supplied any plant material, and their densities were monitored by removing emerged beetles from the cages every 1-2 days. We were thus able to estimate the peak of the emergence to maximize the number of beetles that could be used simultaneously in the experiment, and eventually obtained close to 1000 beetles that had emerged within the same three-day period.

On July 20, 26-28 beetles were transferred into each of the 35 cages, randomly scattered within and around the monoculture patch. Because it is difficult to determine the sex of the beetles before the females become gravid, we could not ensure the same sex ratios in all the cages; however, from preliminary observations, the sex ratio at emergence is roughly 50:50, and the number per cage was sufficient to make it unlikely that a given cage had no or very few females. After 10 days, all live beetles were collected and stored individually in vials filled with ethanol for dissection. Feeding damage was measured on each plant by counting the characteristic feeding holes. This metric was then converted into consumed leaf biomass (dry weight), as described above.

All beetles were dissected and sexed under a dissecting microscope. Unmated females, without developing eggs in their ovaries, were distributed more or less randomly among the host species and cages, and were excluded from further analysis. Eggs inside each mated female were counted to obtain a short-term measure of realized fecundity. Because *Trirhabda* oviposit multiple times throughout their lifetime (mid July until first frost in the study region) and broadcast their eggs singly on the soil and litter (Messina 1982), it is not possible to estimate individual lifetime fitness for a group of females. The short duration of the experiment allowed most females to mate and develop their first batch of eggs, but not to lay it and begin producing their second batch (as verified by dissections), thereby ensuring that our measurements were consistent among individuals.

Data analysis

Field patterns of host use by *Trirhabda* larvae were analyzed using the non-parametric Kruskal-Wallis one-way analysis of variance, with the number of larvae per ramet as the dependent variable. All experiments, except for the no-choice

experiment with larvae, involved groups of insects released into mesocosms of replicated host plants (rather than individual releases of the insect); therefore, host plants could be treated as independent from one another, analogous to a common garden setting. Larval preference (number of larvae per plant) and feeding damage (removed percent leaf area) in greenhouse mesocosms were compared among the seven hosts using the non-parametric Kruskal-Wallis one-way analysis of variance, with mesocosm included as a blocking factor. One-way ANOVA was used to compare relative growth rate of larvae on the seven host species in the no-choice growth chamber experiment, with the Tukey-Kramer HSD test used for post-hoc contrasts among the hosts. Relative growth rate was calculated as the log difference between the final and initial larval weight.

The same statistical approach as with larval preference (above) was taken to analyze adult preference in the cage mesocosms, using the number of beetles per plant and feeding damage (consumed leaf biomass) as the dependent variables. Differences in adult performance on caged monoculture mesocosms of the seven host species were tested using one-way analysis of variance (ANOVA) by comparing short-term individual fecundity (number of eggs per mated female, log-transformed to meet ANOVA assumptions), with cage as the blocking factor. Feeding damage (consumed leaf biomass) in this experiment was compared in a one-way ANOVA, with maternal line and cage nested within host, included in the model. For both fecundity and feeding damage, the Tukey-Kramer HSD test was used for multiple comparisons of treatment means. JMP statistical software (version 7.0, SAS Institute Inc) was used for all analyses.

RESULTS

Larval survey

In the survey of larval distribution at a non-outbreak population density, the number of larvae per ramet differed significantly among the seven hosts ($\chi^2 = 124.68$, df = 6, $p < 0.0001$), with the highest attack rate on *So. altissima* (mean ± SE: 1.76 ± 0.24 larvae/ramet) and *So. rugosa* (0.56 ± 0.11 larvae/ramet), whereas the asters and E. *graminifolia* experienced similar, low attack rates (0.07 ± 0.04 to 0.29 ± 0.07 and 0.16 ± 0.05, larvae/ramet, respectively) (Figure 3.1). Across all the hosts, 24% of ramets had at least a single larva; in contrast, 65% of *So. altissima* ramets had at least

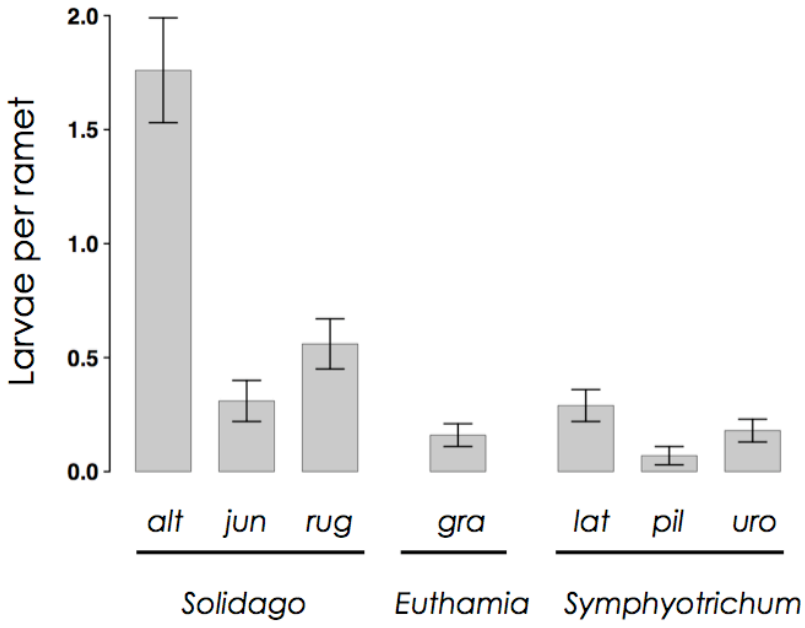

Figure 3.1. Distribution of the larvae of *Trirhabda virgata* (Chrysomelidae) among seven co-occurring host species measured as the mean number of larvae per ramet (n = 71 to 90 per species) ± standard error. Survey conducted a low population density at Whipple Farm, central NY state, in June 2007. Host species: *Solidago* (goldenrods): alt = *So. altissima*; jun = *So. juncea*; *Euthamia*: gra = *E. graminifolia*; rug = *So. rugosa*; *Symphyotrichum* (asters): lat = *Sy. lateriflorum*; pil = *Sy. pilosum*; uro = *Sy. urophyllum*.

one larva, and multiple larvae per ramet were much more common on this host than on the other hosts. Two of the goldenrods (*Solidago altissima* and *So. rugosa*) are the most dominant of the seven hosts at this site, while *Symphyotrichum pilosum* and *Sy. urophyllum* are the rarest, at densities lower by several orders of magnitude.

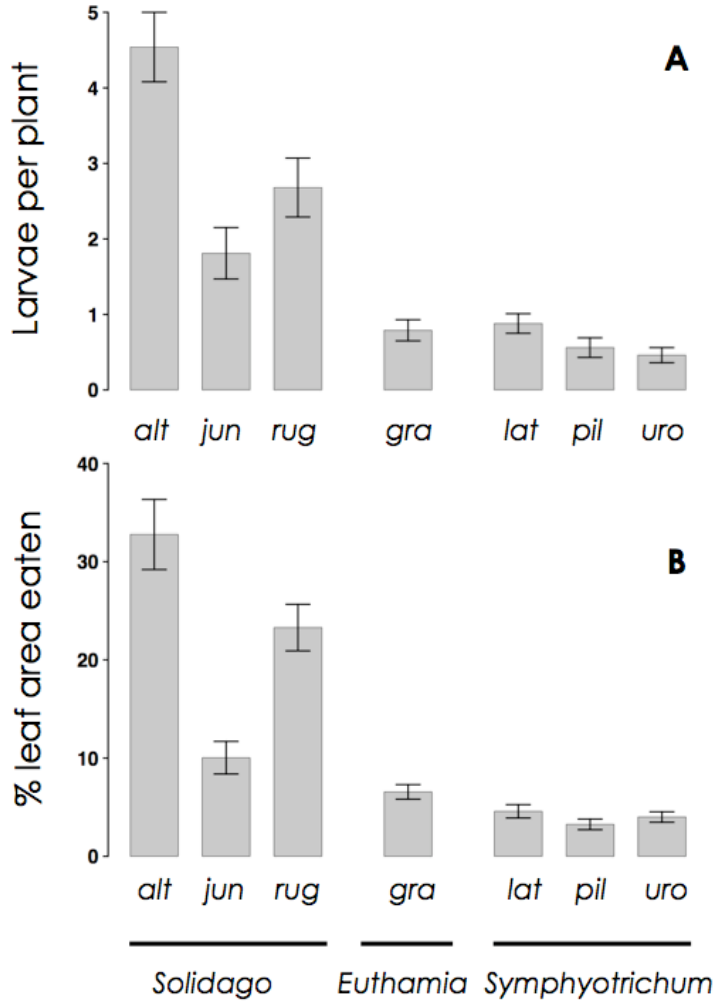

Figure 3.2. Preference of the larvae of *Trirhabda virgata* (Chrysomelidae) in a mesocosm choice experiment with groups of larvae (30) released into ring arrays (n = 16) of seven co-occurring host species (3 plants per host species), shown as A) the mean number of larvae per plant ± standard error; B) the mean percent leaf area eaten per plant ± standard error. Host species: *Solidago* (goldenrods): alt = *So. altissima*; jun = *So. juncea*; *Euthamia*: gra = *E. graminifolia*; rug = *So. rugosa*; *Symphyotrichum* (asters): lat = *Sy. lateriflorum*; pil = *Sy. pilosum*; uro = *Sy. urophyllum*.

Larval preference (choice)

In the greenhouse choice experiment with mesocosm arrays of all seven hosts, the larvae showed a strong hierarchy of host preference ((χ^2 = 119.25, df = 6, p < 0.0001; Figure 3.2A), as most larvae chose to feed on *So. altissima* (4.54 ± 0.46 larvae/plant) or *So. rugosa* (3.75 ± 0.39 larvae/plant), and, to a lesser degree, on *So. juncea* (1.81 ± 0.34 larvae/plant). The extent of feeding damage mirrored these differences (χ^2 = 154.16, df = 6, p < 0.0001; Figure 3.2B): *So. altissima*, *So. rugosa*, and *So. juncea* lost, on average, 32.8 ± 3.6 %, 23.3 ± 2.4%, and 10.0 ± 1.7% of their leaf area, respectively, with much lower feeding damage (4.0-6.6%) on the other four hosts.

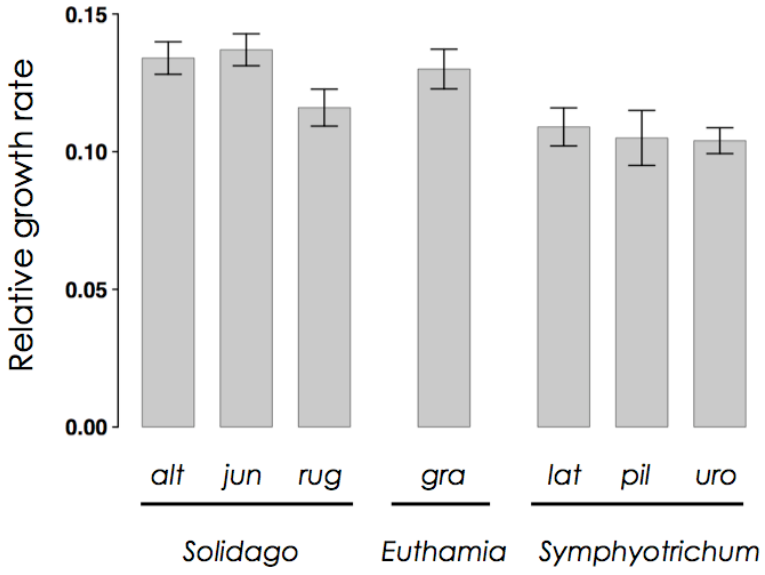

Figure 3.3. Performance of the larvae of *Trirhabda virgata* (Chrysomelidae) feeding singly on potted plants in a no-choice setting (n = 9 to 13 per host), shown as the mean relative growth rate (natural log difference between final and initial weight per time; measured in mg mg^{-1} d^{-1}) ± standard error. Host species: *Solidago* (goldenrods): alt = *So. altissima*; jun = *So. juncea*; *Euthamia*: gra = *E. graminifolia*; rug = *So. rugosa*; *Symphyotrichum* (asters): lat = *Sy. lateriflorum*; pil = *Sy. pilosum*; uro = *Sy. urophyllum*.

Larval performance (no-choice)

Overall, larval performance on potted plants differed significantly across the seven hosts ($F_{6, 75} = 3.83$, $p = 0.0022$), but the differences were not very pronounced, with the range of relative growth rates varying only by 28% (Figure 3.3). Relative growth rate was significantly higher on *So. juncea* (0.137 ± 0.0058 mg mg^{-1} d^{-1}) than on *Sy. pilosum* (0.0105 ± 0.012 mg mg^{-1} d^{-1}) or *Sy. urophyllum* (0.0104 ± 0.018 mg mg^{-1} d^{-1}), with the remaining four hosts showing similar, intermediate growth rates (0.116 to 0.134 mg mg^{-1} d^{-1}).

Adult preference (choice)

In the choice experiment with caged mesocosms, adult beetles clearly preferred *So. altissima* over other host species ($\chi^2 = 26.13$, df = 6, $p = 0.0002$), with 3.6 ± 0.65 beetles per plant (Figure 3.4A). *So. juncea* was moderately preferred (1.42 ± 0.47 beetles/plant), while the other species were chosen only minimally (0.17 to 0.58 beetles/plant). This strong hierarchy of preference was reflected in the significant differences in the amount of feeding damage by the beetles ($\chi^2 = 42.05$, df = 6, $p < 0.0001$). *So. altissima* suffered by far the most damage in terms of removed leaf biomass (116.2 ± 15.5 mg per plant), compared to 11.0 – 36.6 mg/plant for the other host species (Figure 3.4B). Interestingly, *Sy. pilosum* ranked second in the amount of feeding damage (36.6 ± 7.7 mg/plant).

Adult performance (no-choice)

In spite of the strong adult preference for *So. altissima* (above), *Trirhabda* beetles fed extensively on all seven host species in the no-choice experiment with caged host monocultures (Figure 3.5A). Still, the hosts differed by over 50% in the amount of feeding they experienced ($F_{6, 251} = 7.99$, $p < 0.0001$), with *So. altissima*

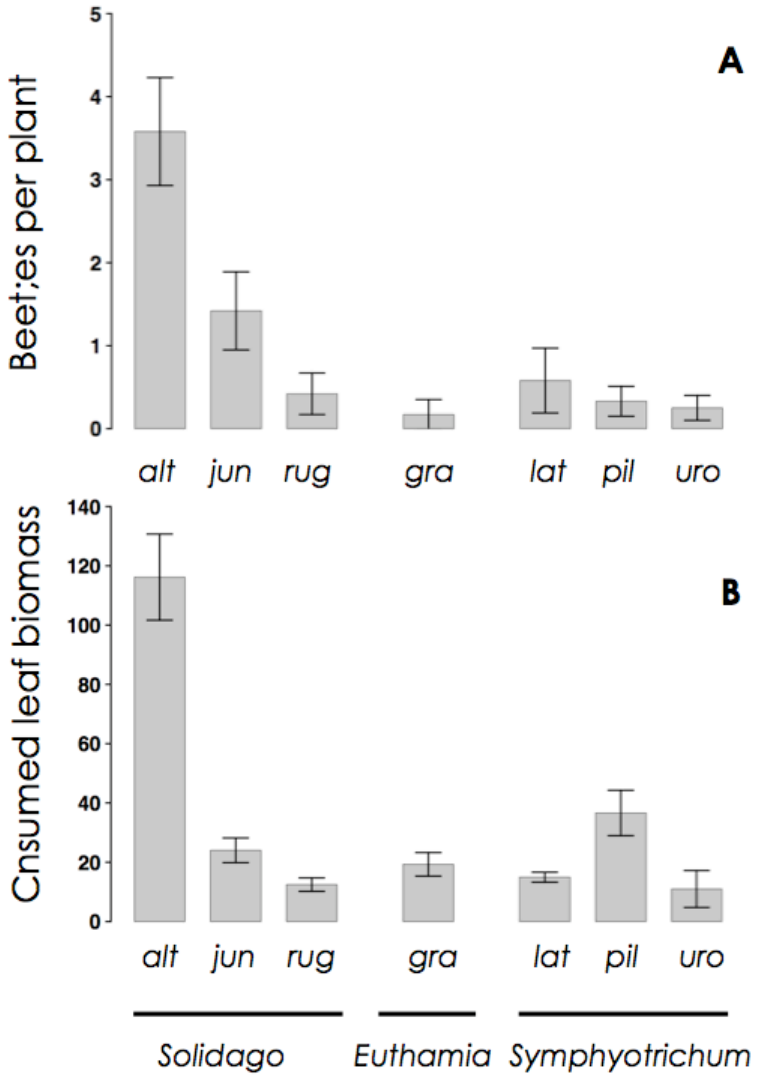

Figure 3.4. Preference of adult *Trirhabda virgata* (Chrysomelidae) beetles in a mesocosm choice experiment with groups of beetles (30) on caged arrays (n = 4) of seven co-occurring host species (3 plants per host species), shown as A) the mean number of beetles per plant ± standard error; B) the mean consumed leaf biomass (dry weight) per plant ± standard error. Host species: *Solidago* (goldenrods): alt = *So. altissima*; jun = *So. juncea*; *Euthamia*: gra = *E. graminifolia*; rug = *So. rugosa*; *Symphyotrichum* (asters): lat = *Sy. lateriflorum*; pil = *Sy. pilosum*; uro = *Sy. urophyllum*.

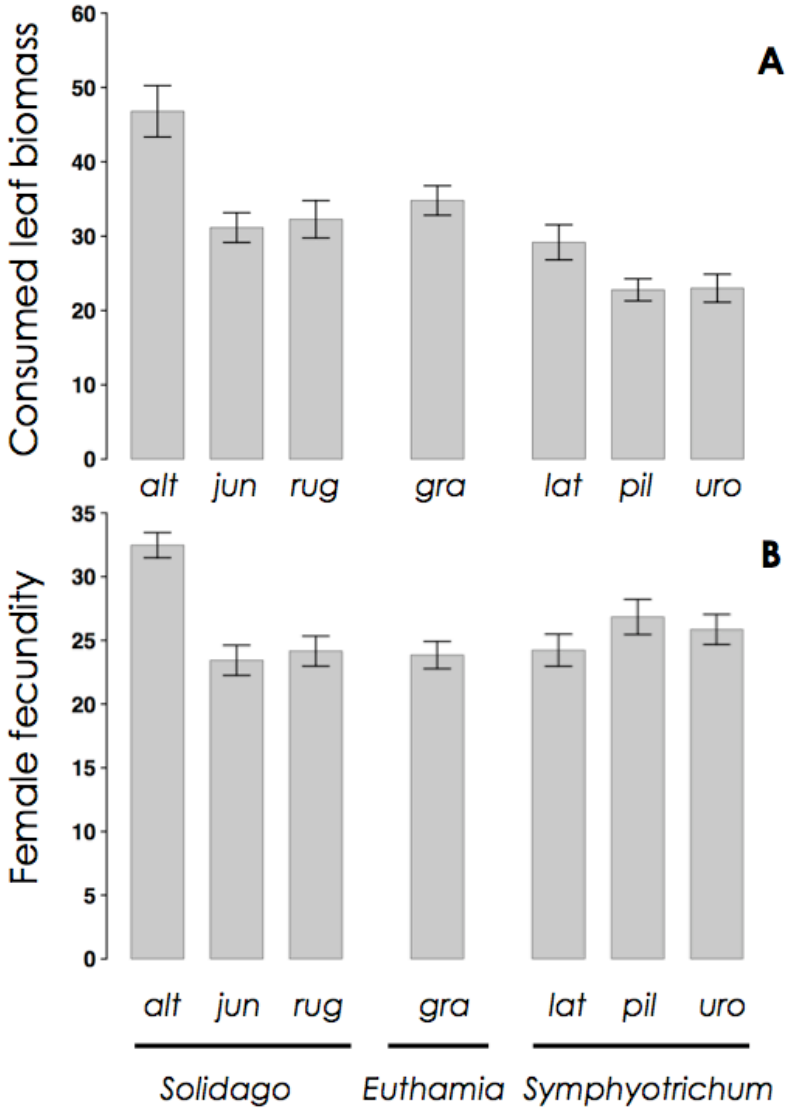

Figure 3.5. Performance of adult *Trirhabda virgata* (Chrysomelidae) beetles after 10 days of feeding on caged monocultures (n = 5) of the seven host species (10 maternal lines per cage, one plant each), showing A) the least squares means of consumed leaf biomass (dry weight) per plant ± standard error, and B) the least squares means of individual female fecundity, measured as the number of developing eggs in the ovaries ± standard error. Host species: *Solidago* (goldenrods): alt = *So. altissima*; jun = *So. juncea*; *Euthamia*: gra = *E. graminifolia*; rug = *So. rugosa*; *Symphyotrichum* (asters): lat = *Sy. lateriflorum*; pil = *Sy. pilosum*; uro = *Sy. urophyllum*.

suffering the greatest damage (46.8 ± 3.6 mg of removed leaf biomass per plant) compared to the other six hosts (22.8 – 32.8 mg/plant). Maternal lines differed significantly in the amount of feeding damage ($F_{63, 251} = 1.59$, $p = 0.0071$), but cages did not ($F_{28, 251} = 0.83$, $p = 0.708$).

The variation in short-term fecundity confirmed *So. altissima* as the host that maximized female performance: egg production was 19% to 39% higher on this host than on the other host species ($F_{6, 224} = 8.49$, $p < 0.0001$), which did not differ significantly from each other (Figure 3.5B). There was a significant effect of cage in this analysis ($F_{28, 224} = 2.11$, $p = 0.0015$), largely due to high variation in the numbers of mated females among cages.

DISCUSSION

Explaining the patterns of host use by phytophagous insects has proven to be challenging as preference hierarchies do not always match insect performance on different hosts. In this study, we investigated the differences in feeding preference and performance of larvae and adults of an outbreaking Chrysomelid beetle, *Trirhabda virgata*, among seven of its common host species, to inform our understanding of the role of life history in host use. Although often studied as a specialist herbivore on *So. altissima* (e.g. Long et al. 2003), *Trirhabda* naturally feeds on a dozen or so related old-field species in the genera *Solidago*, *Symphyotrichum*, and *Euthamia*. In our survey of the seven common hosts in central New York state, we found the highest larval densities by far on the dominant *So. altissima*, followed by the other two goldenrods (*So. rugosa* and *So. juncea*), with lower densities on the other four hosts. This hierarchy of larval distribution is fairly similar to the patterns of relative host abundances in old-fields in this region. It is important to note that this survey captured

larval distribution at a non-outbreak population density. During outbreaks, the larvae tend to defoliate their preferred hosts and spill over onto other suitable hosts (M.S., pers. obs.), likely attenuating eventual differences among the hosts in attack rates and the extent of defoliation. We might therefore expect that, as the outbreaking stage of this insect, the larvae should primarily attack the host species that optimizes their performance and/or is the most abundant, but also that their performance should not be dramatically reduced on other hosts.

Larval preference and performance

Following our predictions, *Trirhabda* larvae showed a strong preference for the dominant *So. altissima* and *So. rugosa* in the mesocosm choice experiment, in agreement with the results previously reported by Messina (1982), and a much lower preference for the asters and *E. graminifolia*. This preference hierarchy is similar to the observed larval distribution among the seven hosts at a low population density, with one notable exception: *So. rugosa* was preferred considerably more than its attack rates in the field would suggest, approaching the level of *So. altissima*. While many factors clearly differ between the field and artificial experimental conditions, one potential explanation is the spillover of larvae from *So. altissima* onto its next preferred host, *So. rugosa*, at higher levels of defoliation, reducing the observed difference between them. Larval preference hierarchy alone may thus explain the natural patterns of larval distribution among the hosts, but it also does not contradict the expected pattern given the relative host abundances.

In contrast with the strong hierarchy of preference when larvae were given a choice, we found weak differences in larval performance on the different hosts in a no-choice setting, with the best and poorest host species differing by only 28% in larval growth rates. *So. juncea* and *So. altissima* provided the highest growth rate, but the

larvae grew nearly as well on most of the other hosts, with only one case (*Sy. pilosum*) of significantly lower larval growth. These findings are similar to those of Messina (1982), who compared larval growth on four *Solidago* species and *E. graminifolia*. Collectively, the performance hierarchy did not contradict the observed strong preference for the primary host, *So. altissima* and, more importantly, it highlighted the ability of the larvae to grow nearly equally well on a variety of related hosts. Such generalized feeding behaviour should be particularly important for early survival of *Trirhabda* larvae, since eggs are not laid on the host plants and the small (2-3 mm) neonates must locate suitable hosts (Messina 1982; see also Marques et al. 1994).

In both choice and no-choice experiments we used larvae that had been previously feeding on the preferred *So. altissima*, rather than naïve, newly hatched larvae with no feeding experience. However, in a preliminary no-choice experiment in which larvae were offered excised leaves of the seven host species, we found no consistent cost of switching from *So. altissima* to another host, in terms of relative growth rate, compared to continued feeding on the previous host species (data not shown). While that experiment did not account for plant induced responses to herbivory, the results add to the evidence that *Trirhabda* larvae can easily make a switch from one host species to another when resources become depleted or reduced in quality, without a marked reduction in their performance (see also Messina 1982). This pattern is in agreement with the prediction of a more generalist strategy in outbreaking insects that are not specialized on a single host species.

Adult preference and performance

Adult performance has been invoked as the critical missing factor to explain the patterns of host use (Scheirs et al. 2000). Specifically, since female fitness is maximized through combined adult and offspring performance, females may prefer to

feed and oviposit on the hosts that may not provide maximum offspring performance but that optimize adult fecundity and lifespan such that overall fitness is maximized. Only recently has this factor of host use been examined, with evidence from several systems, such as the Dipteran grass miner *Chromatomyia nigra* (Scheirs et al. 2000). Given that *Trirhabda* females continue feeding during the period of egg development and oviposition (July – September), we predicted that adult performance should be an important factor in the patterns of adult feeding preference. Furthermore, because the mobile *Trirhabda* oviposit on the soil and plant litter, rather than on the host plants, the relationship between adult and larval host use should be even more decoupled (although, oviposition is presumably most concentrated in patches where adults spend most of their time foraging and mating). Female *Trirhabda* should then preferentially feed on hosts that maximize their lifetime fecundity.

Indeed, we found that adult beetles showed a strong preference for the dominant host, *So. altissima*, and this preference hierarchy was closely matched by differences in adult performance among the hosts, measured as short-term egg production. Therefore, optimization of female performance appears to drive adult tendency towards monophagy in this insect. Although our measure of fecundity cannot incorporate long-term effects of host quality on egg production, or provide information about their impact on female longevity, it may represent a conservative estimate if feeding on the preferred host enhances female longevity. In a multi-year study that included two of the same host species (*E. graminifolia* and *Sy. lateriflorum*), Blatt et al. (1999) found that under indoor conditions, the longevity of *Trirhabda* beetles did not differ but fecundity was higher on the close relative of *So. altissima*, *So.* canadensis, compared to the other two hosts. Our results contradict those of Messina (1982), who found that *Trirhabda* achieved similar lifetime fecundity (and also longevity) on four *Solidago* species and *E. graminifolia*; however, it is possible

that the lack of a difference in fecundity was due to the use of cut plants in that experiment, barring any effects of plant induced responses that have been shown to be important in this system (Wise et al. 2006), and were implicitly included in our experiments. The relative mobility of *Trirhabda* adults compared to the larvae should allow greater selectivity in feeding and little need to feed on suboptimal hosts. Yet, we found that the beetles can still successfully feed on the other host species, albeit at the cost of a 16-28% reduction in egg production, depending on the host. Interestingly, the hosts differed considerably in the amount of feeding they experienced in the no-choice experiment with host monocultures, with *So. altissima* and the largely non-preferred *E. graminifolia* showing the greatest feeding damage. Unfortunately, we could not directly relate the differences in fecundity among the hosts to the differences in the amount of feeding, due to the variation in the survivorship (similar among the hosts) as well as the sex ratio among the cages.

Synthesis

Our study presents a comprehensive examination of the patterns of host use in an outbreaking oligophagous insect, including a broader range of host species than previous investigations in this system. Importantly, it is one of few studies to date that have considered adult performance as an explicit component of the preference-performance relationship (see also Messina 1982, Scheirs et al. 2000). In agreement with the specific life history characteristics of *Trirhabda virgata*, which could also apply to many synovigenic, phytophagous Coleoptera that do not oviposit directly on their host plants (e.g. Marques et al. 1994), we found a more generalist host use by the larvae, contrasted with a much more specialized preference hierarchy of adults, linked strongly to female fecundity. Both life stages preferred the same host, *So. altissima*; this host is by far the most dominant in old-field communities in central New York

state, and has been the focus of numerous studies on plant-herbivore interactions and population dynamics of *Trirhabda virgata* (Root & Cappuccino 1992, Root 1996, Uriarte 2000, Wise et al. 2006); however, very few studies have included other, common, co-occurring hosts (but see Messina 1982, Blatt 1999). In spite of the wealth of information, our understanding of the factors that lead to larval outbreaks, which tend to be regionally asynchronous and highly localized (Root & Cappuccino 1992), still remains incomplete. While it is unlikely that the presence of other host species is essential to trigger population outbreaks, it is possible that it buffers against larval starvation and mortality in the events of extreme local defoliation of *So. altissima*, especially in spatially heterogeneous communities in which *So. altissima* tends to be interspersed among its relatives (Messina 1982). In contrast, adult beetles are unlikely to face depletion of their preferred host, which, by the time of adult emergence, has doubled or tripled in foliar biomass. Adults occur at much lower densities, are much more mobile than the larvae, and tend to disperse to other sites after emergence (Herzig 1995). It is therefore even more critical for the beetles to feed on *So. altissima* in order to maximize their lifetime fecundity, presumably a necessary precondition to build up egg densities that could facilitate a larval outbreak the following year. Understanding the stage-specific patterns of host utilization by *Trirhabda* may thus help explain its eruptive population dynamics, and local coexistence of closely related goldenrod and aster species.

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CHAPTER 4

The role of species relatedness in the feedbacks between insect herbivory and plant community structure

ABSTRACT

Patterns of herbivory may depend on small-scale variation in plant community structure such that plants can receive lesser or greater damage (i.e., benefit from associational resistance or susceptibility, respectively) depending on their neighbours. Herbivores, in turn, may reduce plant growth and alter competitive dynamics, facilitating a change in the community structure. We have investigated this largely unexplored feedback between plant community structure and insect herbivory in a multi-year, field mesocosm experiment with closely related, co-occurring Asteraceae and their outbreaking leaf beetle *Trirhabda virgata*. We constructed equal-density congeneric communities composed of three *Solidago* spp. (goldenrods) or three *Symphyotrichum* spp. (asters), or mixtures of the two genera, and created *Trirhabda* outbreaks in the second and third year in half of the mesocosms, while protecting the other half from all herbivores. Herbivory by *Trirhabda* larvae varied dramatically depending on the community type, with pattern being consistent with the larval host preference hierarchy. Specifically, the preferred goldenrods received greater damage when growing in mixtures with the less preferred asters (i.e., experienced associational susceptibility) compared to congeneric goldenrod stands. In contrast, the less preferred asters received less damage in mixtures, where they enjoyed associational resistance, compared to congeneric stands. Consequently, the mixtures suffered a greater amount of total (across-species) herbivory than would be expected given the damage levels in

the congeneric communities. However, in spite of two years of differential herbivory that depended on the initial community structure, we did not observe a significant shift in the relative performance and competitive abilities of the species due to herbivory, suggesting that the plants countered the impacts of insect herbivores by compensation and tolerance. Nonetheless, insect herbivory reduced overall community productivity and promoted colonization of the mesocosms by other old-field species. Our findings emphasize the role of herbivores in structuring plant communities, while underscoring the reciprocal influence of community structure on the patterns of herbivory, and their potential feedbacks over longer time scales.

INTRODUCTION

As consumers of primary producers, herbivores can have important impacts on the structure of plant communities by selectively removing biomass, reducing individual fitness, and altering competitive dynamics among the species (Brown & Gange 1992, Root 1996, Crawley 2009, Coupe & Cahill 2003, Scherber et al. 2010, but see Hairston et al. 1960). Conversely, the patterns and intensity of herbivory may be influenced by the quality, quantity, and distribution of their food resource, i.e. the variation in plant community structure (Atsatt & O'Dowd 1976, Agrawal et al. 2006). In fact, the feedback between these two processes may be key in understanding the dynamics of plant and herbivore communities, plant coexistence, and local maintenance of diversity (Carson & Root 2000, Schmitz 2008). Yet, the reciprocal effects of plant community structure on patterns of herbivory, and the role of herbivores in shaping the structure of plant communities, have rarely been considered simultaneously (Stein et al. 2010, Scherber et al. 2010).

From a herbivore's perspective, the world comes in many shades of green; plants vary in their suitability as food, having evolved a diverse array of chemical and physical traits to deter or resist the attack by a variety of herbivores (Fraenkel 1959, Ehrlich & Raven 1964, Fritz and Simms 1992, Futuyma & Agrawal 2009). Plant species and even genotypes within a species often differ dramatically in the qualitative and quantitative levels of these defensive traits (Fritz & Simms 1992, Becerra & Venable 1999), producing large variation in plant attractiveness and palatability to a given herbivore (Bernays & Chapman 1994). In a typical plant community, herbivores are thus effectively presented with a complex mixture of more or less suitable (or unsuitable) hosts. Moreover, the likelihood and amount of herbivory on a given plant species often depend on other plant species growing around it (Agrawal et al. 2006, Barbosa et al. 2009). This context-dependence of attack has been recognized primarily in the form of associational resistance: a highly palatable plant may escape attack by a herbivore when surrounded by other, less palatable or unsuitable species (Barbosa et al. 2009), for instance due to the reduced likelihood of visual or olfactory detection by a foraging herbivore (Hamback et al. 2000). However, the opposite situation may also occur: a less preferred host may be more likely to be attacked when growing in the vicinity of a highly preferred host. Such cases of associational susceptibility, although less frequently documented, may occur especially when the more attractive and preferred host is depleted, or mounts an induced defense response, causing a spillover of the herbivores onto the less preferred host (White and Whitham 2000). Ultimately, both associational resistance and susceptibility are mediated by the structure of the plant community, with important consequences for variation in the levels of herbivory.

Whether herbivores actually impose top-down regulation on plant communities has been a matter of a long-standing discussion (Hairston et al. 1960). Herbivory can cause a substantial reduction in plant growth, fitness, and abundance (Crawley 1989,

Schmitz 2008, Crawley 2009). The negative impact of herbivory on individual plants can involve direct effects via plant tissue loss due to consumption and damage, and indirect effects, such as changes in the susceptibility to pathogens (Daleo et al. 2009, but see Hatcher & Paul 2000), and reduced competitive ability against other plants (Hamback & Beckerman 2003). In fact, herbivory may alter the dynamics of plant competition, for instance, via suppression of competitively dominant but less herbivore-resistant species (Lubchenco 1978, Schadler et al. 2010, Scherber et al. 2010). Altered competitive dynamics may thus promote otherwise competitively subordinate species, potentially resulting in increased community evenness and species richness. Such shifts in community structure have been shown particularly in outbreaking insect herbivores (Carson & Root 1999, Carson & Root 2000), or following an intentional or accidental introduction of a herbivore species in the community (Martin et al. 2010). However, plants may also possess the ability to compensate for, or tolerate, damage by herbivores (McNaughton 1983, Trumble et al. 1993, Rosenthal & Kotanen 1994, Strauss & Agrawal 1999), thereby mitigating the negative, top-down effects of herbivory on their performance and competitive interactions with other plants. Impacts of herbivory on plant community structure may therefore not be accurately predicted from the patterns of damage.

Although herbivory and plant competition have usually been considered separately (but see Gurevitch et al. 2000, Hamback & Beckerman 2003, Scherber et al. 2010), we propose that their interactive effects on community structure can be unified via a common conceptual framework that focuses on species relatedness (see also Cavender-Bares et al. 2009). Due to having a more recent common ancestor, closely related species tend to be phenotypically and ecologically more similar than more distantly related taxa (Harvey & Pagel 1991). This similarity may be reflected both in the patterns of herbivory among them, and their competitive interactions. First, close

relatives tend to share many herbivore species (Gilbert & Webb 2004, Weiblen et al. 2006, Pearse & Hipp 2009), especially because a herbivore species adapted to the defensive and nutritional traits of one host species is more likely to be also able to utilize another species with a similar phenotype (Ehrlich and Raven 1964; Agrawal 2007, Futuyma and Agrawal 2009). In plant communities that contain close relatives, patterns of herbivory may then be particularly contingent on the community structure of these co-occurring species. Second, a long-standing but rarely tested hypothesis predicts that, due to their similarity, competition for resources should be more intense among close relatives than among more distantly related taxa (Darwin 1859, Cahill et al. 2008, Valiante-Banuet & Verdu 2008). Consequently, close relatives should competitively exclude each other and rarely co-occur (Harper et al. 1961). Yet, closely related species frequently co-occur in, and sometimes even dominate some communities, such as the Asteraceae-dominated, herbivore-rich open successional habitats ("old fields") of eastern North America. Given this observation, is it possible that differential herbivory on closely related species mediates their local coexistence?

We tested the reciprocal effects of plant community structure and insect herbivory in a multi-year field mesocosm experiment that manipulated plant relatedness. The study included seven closely related, naturally co-occurring, perennial goldenrods and asters (Asteraceae) that dominate old-field communities in central New York state (USA). The mesocosm communities were either protected from insect herbivores with the use of an insecticide, or exposed to two consecutive outbreaks of a dominant outbreaking chewer, *Trirhabda virgata* (Chrysomelidae), that utilizes all the seven hosts. To investigate the role of plant community structure, and specifically, relatedness, on the patterns of herbivory, we compared whether species growing in communities composed of close relatives (congeners) experienced greater herbivory, due to associational susceptibility, than in communities containing a

mixture of the two dominant genera. In turn, we also tested whether the relative performance of each species, and hence the structure of the plant community, shifted due to the *Trirhabda* outbreaks, relative to the herbivore-protected communities, and whether this shift depended on community relatedness. Specifically, differential herbivory may be expected to have stronger impacts in congeneric communities, particularly if competitive interactions are stronger among close relatives, than in mixtures. Finally, we examined the role of feedbacks between herbivory and plant community structure on the invasion of the experimental communities by other, old-field plant species; suppression of the dominant, focal species by insect feeding may facilitate the establishment of other species, and this effect may vary with the initial community structure. Thus, using the framework of community relatedness, we present a novel test of the nature and mechanisms of feedbacks between plant community structure and herbivory.

METHODS

Study system

We selected two locally dominant genera from the family Asteraceae: *Solidago* (goldenrods) and *Symphyotrichum* (New World asters; hereafter, asters). Each genus was represented by three of the most common species that frequently co-occur in central New York state, USA, down to the scale of microsites (M.S., pers. obs.): *Solidago altissima*, *So. juncea*, and *So. rugosa*, and *Symphyotrichum lateriflorum*, *Sy. pilosum*, and *Sy. urophyllum*. In our experimental design, we also included another common and ecologically similar species from a genus more closely related to (and previously included in) the genus *Solidago*, *Euthamia graminifolia*. All seven species also occur naturally at our experimental site (see below).

The focal insect herbivore in our study, *Trirhabda virgata* (hereafter, *Trirhabda*), is a univoltine Chrysomelid leaf beetle whose larvae and adults can be found feeding on the foliage of goldenrods and asters. Known primarily as a specialist on *Solidago altissima*, it naturally feeds on all seven plant species in our study, as well as several other *Symphyotrichum* spp., even when the primary host has not been depleted (M.S. pers. obs.). However, both larvae and adults display a strong preference for S. *altissima* and other goldenrods species over asters, although larval performance on the different hosts does not vary strongly (M.S., unpublished data). The larvae tend to be the most damaging life stage of the insect, as the adults disperse after emergence and their densities tend to be lower by several orders of magnitude (Herzig 1995). During outbreaks in May and early June, the larvae can reach densities of up to 15-30 individuals per stem (M.S. pers. obs.), and can completely defoliate plants. The adults continue feeding for the remainder of the summer but their damage tends to be much lower.

The study was conducted in an old field near Ithaca, central New York state, USA ("North Whipple"; 42°29'32.1"N, 76°25'41.2"W). Previously used for agriculture, the field was abandoned over 40 years ago (R. Root, pers. comm.), and is close to a site where a series of studies on herbivory and old-field plant community dynamics have been conducted since 1970s ("Whipple Farm", e.g. Carson & Root 2000). The field is similar to other old fields in the region, dominated by the native, perennial Asteraceae (esp. *So. altissima*), and is mowed every 3-6 years to prevent the encroachment of woody species. The site had last been mowed about 10 months prior to the beginning of the study (M.S., pers. obs.).

Experimental setup and design

In May 2007, 120 circular holes (app. 90 cm diameter, 30 cm depth) were drilled with a mechanical auger in 40 rows in a checkerboard pattern (with 3 mesocosms per row), spaced out in an area of 200 m x 40 m to capture the natural variation in soil moisture, nutrients, and other abiotic conditions. The soil was partially removed manually, such that a strip of aluminum flashing (app. 30 cm wide and 3 m long) could be inserted vertically along the walls of each mesocosm, extending 22-27 cm below and 3-8 cm above the soil surface. The flashing thus created a circular barrier around the perimeter of each mesocosm, preventing encroachment of other vegetation via below-ground parts, and confining the experimental communities. All soil was then returned into the mesocosms. Each mesocosm was surrounded by a wire fence (app. 2 x 2 m, 1.3 m height) to prevent herbivory by deer and rabbits.

All seed material used to produce plants for this study came from old fields within 1-2 km distance from our experimental site. Seeds were collected from 20-40 individuals per species, that were growing at least 15 m apart to minimize the probability of repeated sampling from the same maternal lines. In April 2007, seeds of each species were pooled from 15-25 maternal plants per species with sufficient germination rates. The seeds were germinated in small groups directly on the moist soil surface (Metro Mix, SunGro Horticulture) in 72-cell trays in the greenhouse, at 13L:11D light regime and 21°C-13°C temperature regime. The seedlings were thinned as needed to ensure that each cell only contained a single plant. The trays were periodically reshuffled to minimize position effects. After 7 weeks, the plants were moved into outdoor cages for several days to acclimate before being transplanted into experimental mesocosms.

Relatedness treatment

In late June 2006, each of the mesocosms was assigned to one of the one of three relatedness treatments, following randomized block design in four blocks. With forty replicate mesocosms in each treatment level, we established two types of congeneric communities (goldenrod congeners or aster congeners) and a mixture of the two genera (hereafter, mixture). The congeneric communities contained three species of the respective genus, with six individual plants per species. The mixtures contained three individuals of each of the six species (i.e. three *Solidago* spp. and three *Symphyotrichum* spp). Finally, in all three types of communities, we also included three individuals of *Euthamia graminifolia*, to act as a phytometer, at the constant proportion of 1/7 of the community. Therefore, all 120 communities started at an equal initial density of 21 individuals, and at species richness of either four species in the congeneric communities of goldenrods or asters (*Solidago* plus phytometer, or *Symphyotrichum* plus phytometer, respectively), or seven species (equal proportion of *Solidago* and *Symphyotrichum*, plus phytometer) in the mixtures. As much as possible, seedlings of similar size were selected for transplanting to reduce size-dependent variation in initial competitive interactions. In the first several weeks after transplanting, mesocosms were watered periodically to aid their establishment, and lightly weeded to prevent fast-growing weeds from outgrowing the focal species. All 2520 plants established successfully and survived the first growing season.

Herbivory treatment

In the first year (2007), all 120 mesocosms were sprayed with a non-systemic insecticide (esfenvalerate, Ortho®, Bug-B-Gon®) once every two weeks during the peak growing season (mid May to mid October) to minimize feeding damage by insect herbivores. Esfenvalerate and a similar insecticide, fenvalerate, have been used in a

number of other studies in this and other systems, and has been found to effectively reduce insect herbivory without phytotoxic effects on plants, and with minimal impacts on soil processes and fertility (Mitchell 2003, and references therein). In the spring of 2008, after one year of insect-free growth, we assigned each of the mesocosms to one of two levels of herbivory treatment: insect herbivory versus insect exclusion. The herbivory treatment was fully crossed with the three levels of relatedness, in a randomized block design, producing 20 replicates per treatment combination. The mesocosms in the insect exclusion treatment continued the insecticide spray application as described above. At the time of each spraying, the mesocosms open to insects received a spray application of an equal amount of water.

To supplement the ambient levels of damage in the insect herbivory treatment, we created consecutive outbreaks of *Trirhabda virgata* in 2008 and 2009 by releasing larvae into the mesocosms. Each year, about 20,000 larvae of mixed ages (with the majority in the 2nd or 3rd instar) were collected from an outbreaking population in an old-field dominated by S. *altissima*, located about 2 km away. An equal number of larvae (about 400) were then released into each of the 60 mesocosms. This number approximates median per-ramet densities during *Trirhabda* outbreaks (M. S. pers. obs.), since the mesocosms contained 60-150 regrown ramets; however, the actual number of larvae per ramet was often lower since the larvae were able to leave the mesocosms and feed on the surrounding vegetation. Depending on their instar, the larvae fed in the mesocosms for a period of 8-15 days prior to their pupation.

Depending on the species, mesocosms open to insect herbivory also experienced feeding damage by other insect herbivores, including leaf rollers, galling insects, spittle bugs, flea beetles, and others. For the remainder of the growing season, *Trirhabda* adults also continued feeding at low densities on the plants in the herbivory treatment. Other than their exclusion from the insect-free treatment, the densities of

these herbivores were not manipulated, and their levels were several orders of magnitude lower than those of *Trirhabda* larvae during the outbreaks.

Data collection

As soon as most of *Trirhabda* larvae had pupated, we surveyed foliar feeding damage on each species by randomly selecting eight ramets per species in each mesocosm. In cases were fewer than eight ramets were available, all ramets were included. The damage was estimated visually as the percent leaf area removed by larval feeding, to the closest 5%. The data were then averaged across the sampled ramets per species to avoid pseudoreplication. In September 2008, when insect herbivores started to decline, we surveyed all mesocosms for foliar feeding damage, following the same procedure as above. This survey represents cumulative herbivory by a variety of chewing insect herbivores; several other feeding guilds were also included (e.g. galling insects) but were not common enough to allow statistical inference. We conducted the same survey in the insecticide treatment, to confirm the efficacy of the insect exclusion treatment.

Every fall, we harvested above-ground biomass of each species once most of its above-ground parts had senesced, so as not to affect the allocation of resources into below-ground parts for regrowth in the next season. All biomass was dried for a minimum of 48 hours at 60ºC, and weighed.

In July 2009, we conducted a survey of all mesocosms for other vegetation that had colonized the experimental communities during the three years. We identified and recorded all the species, and estimated their individual percent cover. In order to accurately capture the vertical complexity of the vegetation, our method allowed for counting the same area two or more times if it was occupied by two or more overlapping species; i.e. the total of percent cover values for all species can add up to

more than 100%. Because of the late senescence and harvest of our focal species, we were unable to harvest and measure the above-ground biomass of the species that have invaded the mesocosms, as much of their biomass would have been lost by then.

Statistical analysis

All of the analyses involved either a univariate or multivariate analysis of variance (or covariance) approach, and were conducted using the free software R (R Development Core Team 2007). While our experiment involved a randomized block design, block was not included in any of the analyses presented here; in preliminary analyses, using mixed effects models (function lmer in R package lme4) with relatedness and herbivory as fixed effects and block as a random effect, block explained very little variance in all cases.

To test the effect of the relatedness of the plant community on the amount of herbivory by *Trirhabda* larvae, we analyzed feeding damage data from the insect treatment only, using two approaches. The first approach focused on herbivory at the community level, using the percent leaf area eaten averaged across all focal species in the community. For each of the two years, we used a simple linear model with relatedness as the main effect, and two a priori, orthogonal contrasts (Bolker 2008): one testing whether goldenrod and aster congeneric communities differed in the amount of herbivory, and the other testing whether the mixtures experienced lower herbivory than would be expected given the feeding damage in the congeneric communities (i.e. test for the difference between the mixtures and the mean of the congeneric communities). A significant interaction between herbivory and each respective contrast would indicate that the effects of the herbivory treatments differed with respect to the specific relatedness comparison. In the second approach, we tested for the effect of relatedness at the level of individual species using MANOVA in order

to account for non-independence of the species within the mesocosms. Because of the design of our experiment, goldenrod congeneric communities do not contain any asters, and vice versa, while the mixtures contain all the seven species. Therefore, we split the analysis into two separate parts: one analysis compared the amount of herbivory on goldenrods (*Solidago* spp.) in their respective congeneric communities versus in the mixtures, while ignoring the asters (*Symphyotrichum* spp.); the other analysis focused on the asters while ignoring the goldenrods. *Euthamia graminifolia* was included in both analyses, since this species was present in all three types of communities. In both cases, percent leaf area removed in each of the four species shared between the two community types (i.e. goldenrods or asters, respectively, and *Euthamia*) represented a multivariate response, and was log-transformed to meet the assumptions of parametric statistics. Using the same approach, we also analyzed percent leaf damage measured in September 2008, to test for the patterns of cumulative herbivory by leaf chewing insects.

We tested whether the three types of communities differed in the impact of herbivory by comparing community productivity (total above-ground biomass) in the insect treatment versus the herbivore-free treatment. Because the mesocosm-level response variables were not significantly correlated between 2008 and 2009, we analyzed the two years separately. We used a linear model with relatedness and herbivory as the main effect, and including their interaction. As in the analysis of community herbivory (above), we set two a priori, orthogonal contrasts, as described above: one testing whether goldenrod and aster congeneric communities differed in the amount of herbivory, and the other testing whether the mixtures experienced lower herbivory than would be expected given the feeding damage in the congeneric communities. When the average percent leaf area removed in each community was included as a covariate in the model, the main effect of herbivory was no longer

significant; we interpret this as evidence that our data on feeding damage accounted for most of the variation in community productivity due to the herbivory treatment.

In order to specifically assess the impact of herbivory on plant community structure, we tested for the effect of the herbivory treatment on individual species performance at the end of the third year, and whether this effect varied depending on the community relatedness. We employed MANOVA, split into two separate parts as described above: a comparison of the biomass of *Solidago* spp. and *Euthamia* between the goldenrod congeneric communities and mixtures, and a comparison of the biomass of *Symphyotrichum* spp. and *Euthamia* between the aster congeneric communities and mixtures. For all species, the dependent variable was standardized for the initial (planted) number of individuals of each species, in order to allow a comparison of the mixtures (three starting individuals per species) and congeneric communities (six starting individuals per species). The multivariate response variable analyzed in MANOVA thus included the standardized above-ground biomass (log-transformed to meet the parametric assumptions) of four species, while ignoring the biomass of the other three present in the mixtures. We focused our analyses on biomass data from 2009, since they represent the cumulative effects of the herbivory and relatedness treatment in our experiment. In a separate, identical MANOVA, we also analyzed 2008 biomass data to check whether the effects of herbivory were more pronounced after the first outbreak.

The effect of herbivory on the invasion of the experimental communities by other old-field species by the third year, and its contingency on the initial community structure (relatedness), were analyzed in a linear model (lm function in R), with herbivory and relatedness as the main effects. The two dependent variables, species richness of invaders and total percent cover of invaders, were analyzed separately, and were log-transformed to improve normality and homogeneity of variances. Total

above-ground biomass of the focal species was included as a covariate but was not significant and was dropped from the model. To test whether goldenrod and aster congeneric communities differed in the impact of herbivory on invasion, and whether the invasion of the mixtures under the two herbivory treatments differed from that predicted based on the invasion of the congeneric communities, we set up a priori, orthogonal contrasts as described above.

RESULTS

At the community level (i.e., entire mesocosms), congeneric communities of goldenrods received significantly more mid-season herbivory by *Trirhabda* larvae compared to those of asters (Figure 4.1, average percent leaf area removed was 90% higher in 2008, t value = 4.939, $p < 0.0001$; and 51% higher in 2009, t value = 5.189, $p < 0.0001$). However, in both years, the mixtures experienced significantly greater feeding damage than expected given the levels of herbivory in the two congeneric communities (contrast of the mean congeneric communities and mixtures: 2008: t value = -2.376, $p = 0.021$; 2009: t value -2.524, $p = 0.014$), suggesting that goldenrods or asters, or both, received more damage when growing in the mixtures.

When examining the levels of feeding damage by *Trirhabda* on individual species, a contrasting pattern of associational resistance versus associational susceptibility emerges in the mixtures (Figure 4.2). Specifically, the more preferred *Solidago* spp. suffered up to 60% higher feeding damage when growing in mixtures than when growing only with congeners (Table 4.1A, MANOVA, overall herbivory effect: Wilks' $\lambda = 0.725$, $F_{1,4} = 3.324$, $p = 0.021$, in 2008, and Wilks' $\lambda = 0.586$, $F_{1,4} = 6.195$, $p = 0.0007$ in 2009); in contrasts, *Symphyotrichum* spp. benefited from up to a 54% reduction in damage when growing with goldenrods (Table 4.1B, MANOVA,

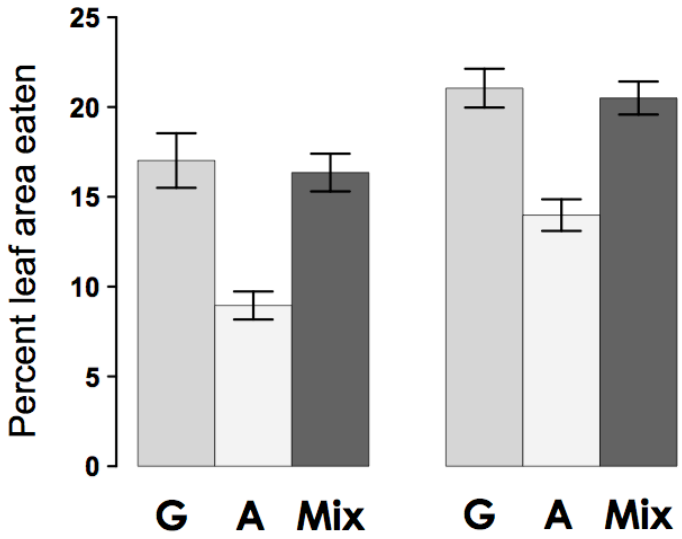

Figure 4.1. Average defoliation (percent leaf area eaten) by *Trirhabda virgata* larvae at the community level, comparing the three relatedness treatments in 2008 and 2009. G = four-species, goldenrod congeneric community; A = four-species, aster congeneric community; Mix = seven-species mixture of goldenrods and asters. Means ± standard errors; n = 20 per treatment.

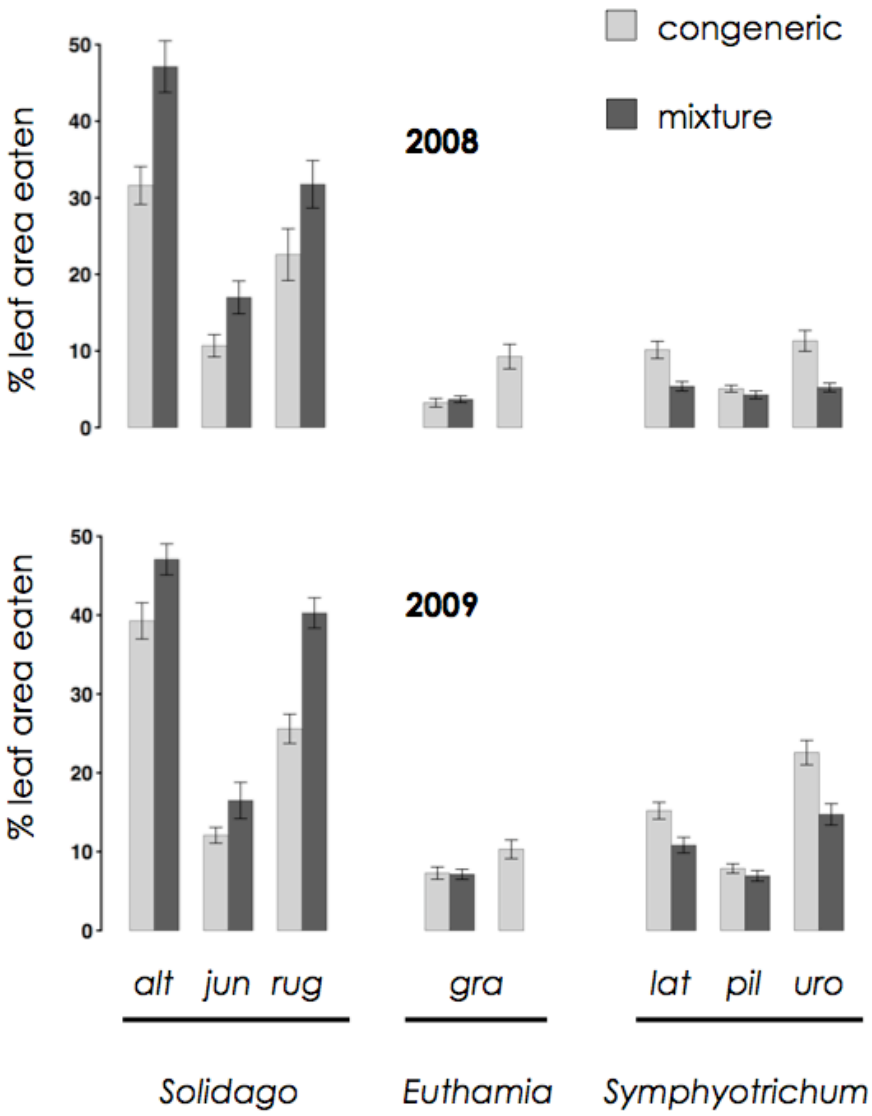

Figure 4.2. By-species comparison of average defoliation (percent leaf area eaten) by *Trirhabda virgata* larvae between the respective goldenrod or aster congeneric communities (light bars) and mixtures (dark bars), following the 2008 (top) and 2009 (bottom) outbreaks. Species abbreviations: alt = *Solidago altissima*; jun = *So. juncea*; rug = *So. rugosa*; gra = *Euthamia graminifolia* (present in all three treatments; light bars on the left and right show herbivory in the goldenrod and aster congeneric communities, respectively); lat = *Symphyotrichum lateriflorum*; pil = *Sy. pilosum*; uro = *Sy. urophyllum*. Means ± standard errors; n = 20 per treatment.

Table 4.1. Summary of multivariate analysis of variance with the average per-ramet defoliation (percent leaf area removed) of each species as the non-independent (multivariate) response variable in each outbreak year (2008 and 2009). For each species, the treatment effect (relatedness) involves a contrast of feeding damage when growing in mixtures versus in congeneric communities with A) goldenrods or B) asters. For each species and year, the univariate analyses are presented below. *E. graminifolia* was planted in all three community types; hence, two contrasts are presented.

Table 4.1

4.1A. Goldenrod congeneric communities versus mixtures.

MANOVA test criteria (Wilks' λ) and F statistics

Overall	Source	Wilks' λ	F	df	p
2008	Relatedness	0.725	3.324	1, 4	0.021 *
2009	Relatedness	0.586	6.195	1, 4	< 0.0001 ***

Univariate ANOVA for species by year:

So. altissima	Source	df	SS	F	p
2008	Relatedness	1	1.626	11.93	0.0014 **
	Error	38	5.177		
2009	Relatedness	1	0.406	6.959	0.012 *
	Error	38	2.214		
So. juncea	Source	df	SS	F	p
2008	Relatedness	1	1.746	4.621	0.038 *
	Error	38	14.36		
2009	Relatedness	1	0.608	3.441	0.071
	Error	38	6.708		
So. rugosa	Source	df	SS	F	p
2008	Relatedness	1	1.967	7.100	0.011 *
	Error	38	10.53		
2009	Relatedness	1	2.480	23.87	< 0.0001 ***
	Error	38	3.949		
E. graminifolia	Source	df	SS	F	p
2008	Relatedness	1	0.382	1.564	0.219
	Error	38	9.281		
2009	Relatedness	1	<0.01	< 0.01	0.995
	Error	38	6.712		

Table 4.1 (Continued)

4.1B. Aster congeneric communities versus mixtures

MANOVA test criteria (Wilks' λ) and F statistics

Overall	Source	Wilks' λ	F	df	p
2008	Relatedness	0.505	8.592	1, 4	< 0.0001 ***
2009	Relatedness	0.652	4.661	1, 4	0.0040 **

Univariate ANOVA for species by year:

Sy. lateriflorum	Source	df	SS	F	p
2008	Relatedness	1	3.710	17.37	0.0002 ***
	Error	38	8.115		
2009	Relatedness	1	1.402	12.03	0.0013 **
	Error	38	4.429		
Sy. pilosum	Source	df	SS	F	p
2008	Relatedness	1	0.486	2.458	0.125
	Error	38	7.513		
2009	Relatedness	1	0.206	1.678	0.203
	Error	38	4.675		
Sy. urophyllum	Source	df	SS	F	p
2008	Relatedness	1	5.858	23.94	< 0.0001 ***
	Error	38	9.298		
2009	Relatedness	1	2.146	16.73	0.0022 **
	Error	38	4.876		
E. graminifolia	Source	df	SS	F	p
2008	Relatedness	1	6.125	18.26	0.00012 ***
	Error	38	12.74		
2009	Relatedness	1	1.109	5.694	0.022 *
	Error	38	7.402		

overall herbivory effect: Wilks' $\lambda = 0.505$, $F_{1,4} = 8.592$, $p < 0.0001$, in 2008, and Wilks' $\lambda = 0.652$, $F_{1,4} = 4.661$, $p = 0.0040$ in 2009). Therefore, the unexpectedly high level of herbivory at the community level in the mixtures (see above, and Figure 4.1) can be attributed to increased levels of attack on *Solidago* spp. Similar results were obtained when *Euthamia* was excluded from the analysis (not shown). However, by the end of the growing season, none of the species showed differences in the cumulative foliar damage between the mixtures and their respective congeneric communities (MANOVA, goldenrods versus mixtures: Wilks $\lambda = 0.928$, $F_{1,4} = 1.413$, $p = 0.238$; asters versus mixtures: Wilks $\lambda = 0.950$, $F_{1,4} = 0.982$, $p = 0.423$).

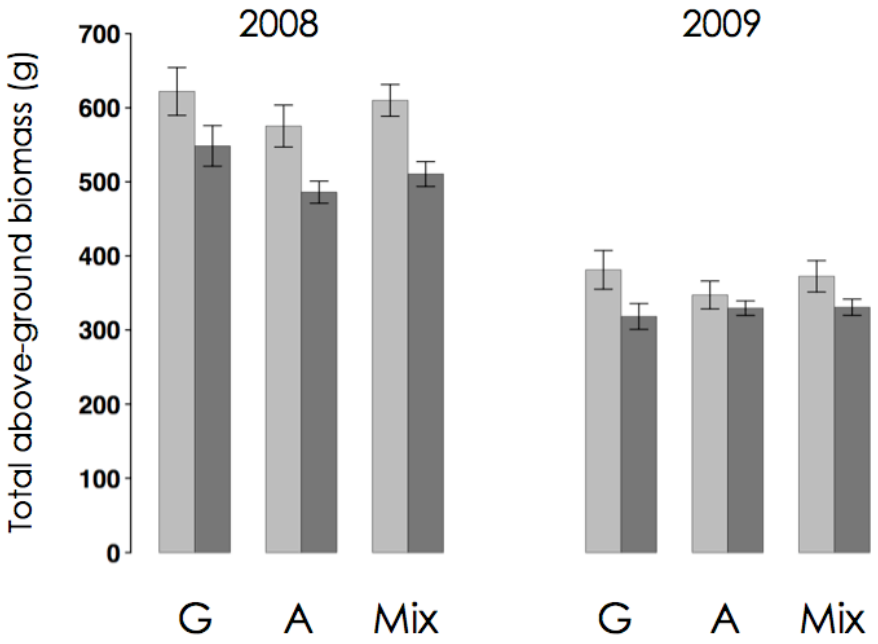

Figure 4.3. Comparison of total community productivity, measured as above-ground biomass at the end of the season, in the three relatedness treatments protected from insect herbivory (insecticide treatment, light bars) versus exposed to insect herbivores (dark bars), in 2008 and 2009. G = four-species, goldenrod congeneric community; A = four-species, aster congeneric community; Mix = seven-species mixture of goldenrods and asters. Means ± standard errors; n = 20 per treatment.

Insect herbivory was associated with an average reduction of 13% in community productivity (above-ground biomass) compared to the communities where insects were excluded (Figure 4.3). This effect was comparable across the three relatedness treatments (ANOVA, herbivory x relatedness: $F_{2, 114} = 0.228$, $p = 0.796$ in 2008; $F_{2, 114} = 0.959$, $p = 0.386$ in 2009), and, surprisingly, was less pronounced after the second year of herbivory (15% average reduction in 2008 versus 11% reduction in 2009), when community productivity was also lower overall. Average community-level damage explained only a very small and non-significant portion of the total variance in productivity ($R^2 = 0.035$ and 0.023 for 2008 and 2009, respectively).

Importantly, in spite of two consecutive bouts of differential herbivory that varied with community relatedness and individual species (Figure 4.1 and 4.2), insect herbivores did not cause a shift in the plant community structure in any of the relatedness treatments by the third year (Figure 4.4, Table 4.2). While biomass (standardized per initial number of individuals) of *Solidago* spp. and *Euthamia* was significantly reduced by herbivory overall (Table 4.2, MANOVA, herbivory effect: Wilks' $\lambda = 0.706$, $F_{1, 4} = 4.219$, $p < 0.0001$), this effect was similar between the congeneric communities and the mixtures (MANOVA, herbivory x relatedness: Wilks $\lambda = 0.928$, $F_{1, 4} = 1.371$, $p = 0.252$), in which these species attained similar biomass (MANOVA, relatedness effect: Wilks' $\lambda = 0.944$, $F_{1, 4} = 1.063$, $p = 0.381$). Likewise, the overall effect of herbivory on the standardized biomass of *Symphyotrichum* spp. and *Euthamia* was similar in the aster congeneric communities and mixtures (Table 4.2, MANOVA, herbivory x relatedness: Wilks' $\lambda = 0.944$, $F_{1, 4} = 1.085$, $p = 0.371$). However, in this case herbivory did not lead to a significant overall reduction in biomass (MANOVA, herbivory effect: Wilks' $\lambda = 0.918$, $F_{1, 4} = 1.632$, $p = 0.175$), although overall the species biomass was significantly higher in the mixtures than in

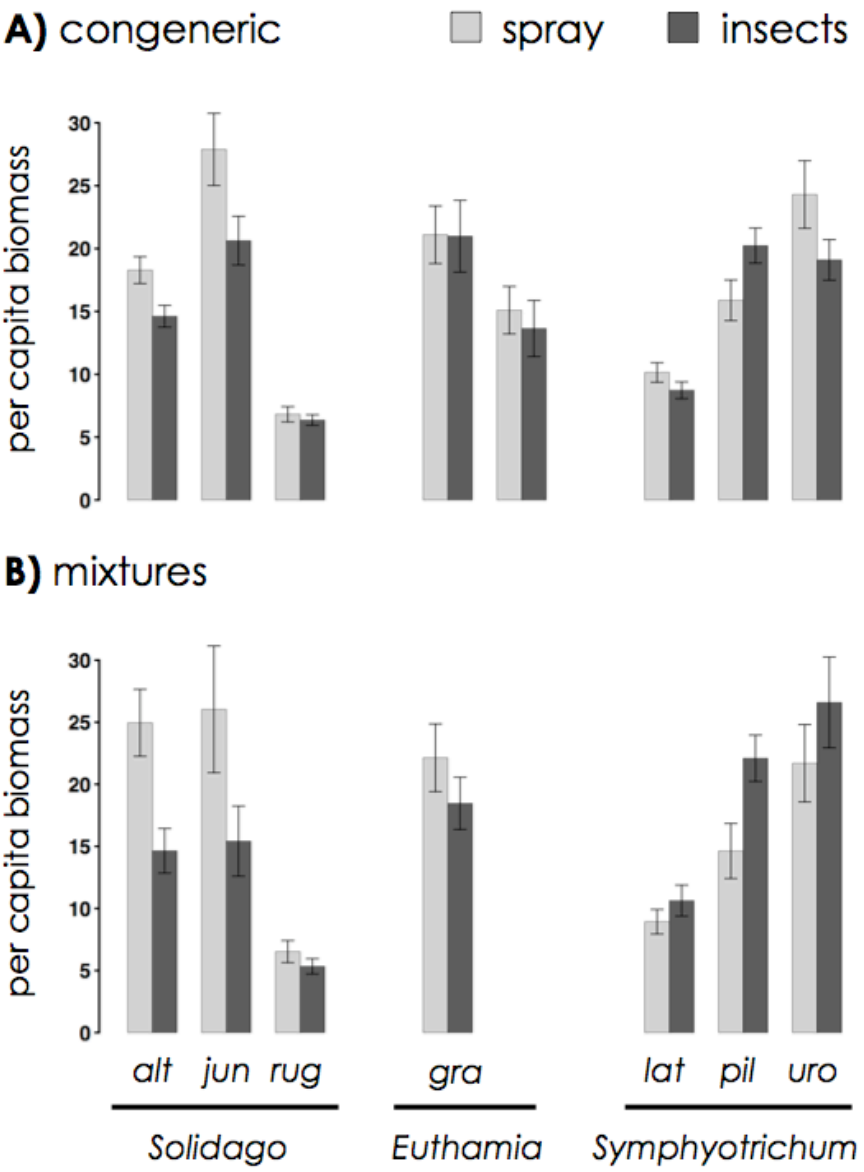

Figure 4.4. Comparison of species performance (above-ground biomass) when protected from insect herbivores (insecticide treatment, light bars) versus when exposed to two seasons of insect herbivory, in their respective goldenrod or aster congeneric communities (top panel) and mixtures (bottom panel). Species abbreviations: alt = *Solidago altissima*; jun = *So. juncea*; rug = *So. rugosa*; gra = *Euthamia graminifolia* (present in all three treatments; in the top panel, left and right pair of bars show performance in the goldenrod and aster congeneric communities, respectively); lat = *Symphyotrichum lateriflorum*; pil = *Sy. pilosum*; uro = *Sy. urophyllum*. Means ± standard errors; n = 20 per treatment.

Table 4.2. Summary of multivariate analysis of variance with the performance of each species (above-ground biomass in 2009, standardized per initial number of individuals) as the non-independent (multivariate) response variable, and herbivory treatments and community relatedness as the main effects, including their interaction term. For each species and overall, the treatment effect of relatedness involves a contrast of the respective (goldenrod or aster) congeneric communities versus mixtures. *E. graminifolia* was planted in all three community types; hence, two separate contrasts are presented. The summaries of univariate analyses for each species are listed below.

Table 4.2

MANOVA test criteria (Wilks' λ) and F statistics

Overall	Source	Wilks' λ	F	df	p
Goldenrods	Herbivory	0.706	7.389	1, 4	< 0.0001 ***
(*Solidago* +	Relatedness	0.944	1.063	1, 4	0.381
Euthamia)	H x R	0.928	1.371	1, 4	0.253
Asters	Herbivory	0.918	1.632	1, 4	0.175
(*Symphyotrichum*	Relatedness	0.867	2.791	1, 4	0.032
+ *Euthamia*)	H x R	0.944	1.085	1, 4	0.371

Univariate ANOVA for species by year:

So. altissima	Source	df	SS	F	p
	Herbivory	1	2.738	12.57	0.00068 ***
	Relatedness	1	0.031	0.141	0.709
	H x R	1	0.706	3.240	0.076
	Error	74	16.11		
So. juncea	Source	df	SS	F	p
	Herbivory	1	2.200	2.463	0.121
	Relatedness	1	2.986	3.342	0.072
	H x R	1	0.114	0.128	0.722
	Error	74	66.10		
So. rugosa	Source	df	SS	F	p
	Herbivory	1	0.034	0.075	0.786
	Relatedness	1	1.000	2.180	0.144
	H x R	1	0.077	0.167	0.684
	Error	74	33.95		
E. graminifolia	Source	df	SS	F	p
(with goldenrods	Herbivory	1	0.423	1.224	0.272
vs. in mixtures)	Relatedness	1	0.011	0.033	0.857
	H x R	1	0.137	0.395	0.532
	Error	74	25.59		

Table 4.2 (Continued)

Sy. lateriflorum	Source	df	SS	F	p
	Herbivory	1	0.999	3.599	0.062
	Relatedness	1	2.073	7.466	0.0078 **
	H x R	1	0.454	1.634	0.205
	Error	76	21.10		
Sy. pilosum	Source	df	SS	F	p
	Herbivory	1	0.325	1.443	0.233
	Relatedness	1	0.777	3.456	0.067
	H x R	1	0.119	0.530	0.469
	Error	76	17.09		
Sy. urophyllum	Source	df	SS	F	p
	Herbivory	1	2.200	2.585	0.112
	Relatedness	1	0.427	0.501	0.481
	H x R	1	0.923	1.085	0.301
	Error	76	64.69		
E. graminifolia	Source	df	SS	F	p
(with asters vs. in mixtures)	Herbivory	1	0.173	1.321	0.254
	Relatedness	1	0.406	3.096	0.083
	H x R	1	0.349	2.661	0.107
	Error	76	9.964		

the congeneric communities (MANOVA, relatedness effect: Wilks' $\lambda = 0.867$, $F_{1, 4} = 2.791$, $p = 0.032$). When *Euthamia* was excluded from the analyses to consider the effects on a single genus only, the results remained similar for *Solidago* spp., but there was a significant effect of herbivory on *Symphyotrichum* spp. (MANOVA, herbivory effect: Wilks' $\lambda = 0.880$, $F_{1, 4} = 3.226$, $p = 0.028$), although the interaction term still remained non-significant (MANOVA, herbivory x relatedness: Wilks' $\lambda = 0.964$, $F_{1, 4} = 0.890$, $p = 0.451$). The individual species varied in their responses to herbivory and the relatedness of the community, but these effects were almost exclusive to the first outbreak (data not shown); by 2009, of all the species only *So. altissima* showed a significant overall impact of herbivory on its biomass (Table 4.2, ANOVA, relatedness effect: $F_{1, 74} = 12.57$, $p = 0.0007$).

Consequently, in only three cases was the interaction term (herbivory x relatedness) significant. In 2008, the effects of herbivory on species biomass varied depending on the community relatedness in only two species: *So. altissima* and *Euthamia* (ANOVA, herbivory x relatedness: $F_{1, 74} = 4.273$, $p = 0.042$, and $F_{1, 74} = 5.114$, $p = 0.027$, respectively). By 2009, only *So. altissima* showed a marginally greater effect of herbivory in the mixtures than in the congeneric communities (Table 4.2, ANOVA, herbivory x relatedness: $F_{1, 74} = 3.240$, $p = 0.076$), suffering a 41% versus 18% reduction in its biomass, respectively, compared to growing protected from insect herbivory.

Insect herbivory was associated with greater invasion of mesocosms by other old-field plant species compared to the insect-exclusion treatment, but this herbivory effect did not vary with relatedness (Figure 4.5, Table 4.3: herbivory x relatedness interaction). On average, communities with insect herbivory were invaded by 10% more species on average than the protected communities (ANOVA: $F_{1, 114} = 4.338$, $p = 0.039$), and the colonizing species were also 14% more abundant in terms of their

total percent cover (ANOVA: $F_{1, 114} = 4.934$, $p = 0.028$). For species richness but not for total percent cover of invaders, the mixtures were more invaded than would be expected from the invasion of the two congeneric communities (contrast of mean congeneric communities and mixtures: t-value = 3.057, p = 0.0028, and t-value = 1.495, p = 0.138, respectively), and in both cases there was an overall effect of relatedness (richness: $F_{1, 114} = 11.62$, $p < 0.0001$; total cover: $F_{1,114} = 3.388$, $p = 0.037$). However, the interaction term of herbivory and relatedness was not significant for either variable (Table 4.3), suggesting that the effects of herbivory on invasibility were similar regardless of the community relatedness.

Table 4.3. Summary of analyses of variance of species richness and total percent cover of invaders, with community relatedness, herbivory, and their interaction included in the model.

Species richness	df	SS	F	p
Relatedness	2	171.65	11.621	< 0.0001 ***
Herbivory	1	32.03	4.338	0.039 *
Relatedness x herbivory	2	3.62	0.245	0.783
Residuals	114			

Total cover	df	SS	F	p
Relatedness	2	1.078	3.388	0.037 *
Herbivory	1	0.785	4.934	0.028 *
Relatedness x herbivory	2	0.221	0.693	0.502
Residuals	114			

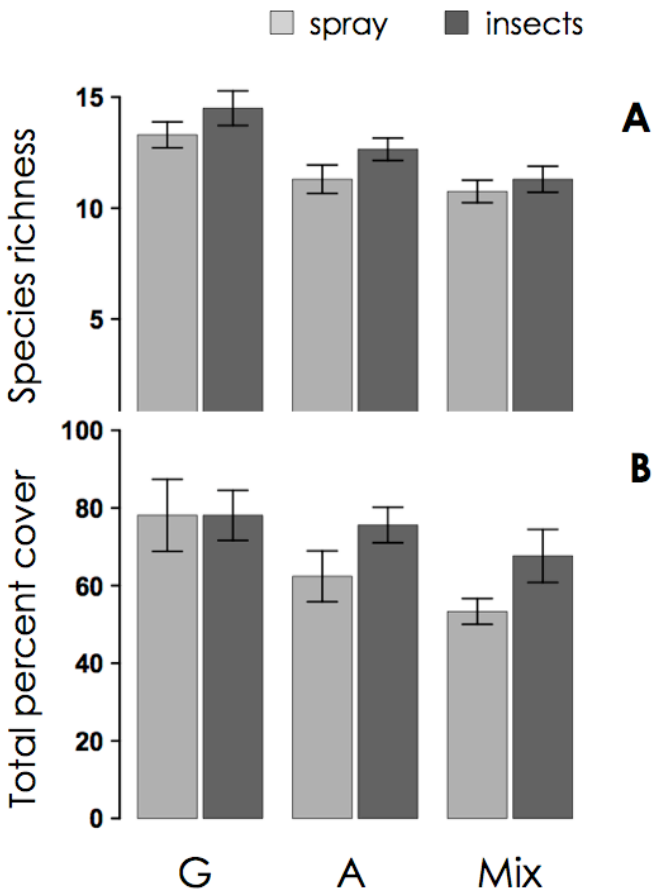

Figure 4.5. Comparison of community invasibility after three years in the absence (light bars) and presence (dark bars) of insect herbivory, among the three relatedness treatments (G = four-species, goldenrod congeneric community; A = four-species, aster congeneric community; Mix = 7-species mixture of goldenrods and asters). A) Species richness of the invaders; B) Total percent cover of the invaders, allowing overlapping cover (i.e. total cover in a community can be greater than 100%). Means ± standard errors; n = 20 per treatment.

DISCUSSION

Plants and herbivores are intricately linked, and their trophic interaction has important cascading consequences for other trophic levels (Schmitz 2008). Yet, our understanding of how the variation in the plant community structure drives patterns of herbivory, and how herbivores in turn shape community structure, is surprisingly incomplete. Our findings from a three-year field mesocosm experiment, in which we manipulated insect herbivory and plant community structure, highlight the potential role of this feedback in the coexistence of closely related plant species and maintenance of local plant diversity.

We presented the evidence that plant community structure can act as a major determinant of the patterns of insect herbivory in dominant old-field Asteraceae. Our manipulation of the relatedness of plant communities created dramatically different levels of herbivory on the individual species by the dominant leaf-chewing insect, *Trirhabda virgata*, which could be explained by the feeding preference hierarchy of this outbreaking broad specialist (M. S., unpublished results). When growing intermixed with more distant relatives, the more preferred goldenrods (*Solidago* spp.) suffered from associational susceptibility, as the insects concentrated their feeding on them and mostly avoided the less preferred asters (*Symphyotrichum* spp.), which benefited from associational resistance in this community context. The pattern was reversed for the two genera in their own congeneric communities: in the absence of the preferred *Solidago* spp., asters suffered higher *Trirhabda* herbivory, whereas congeneric stands of goldenrods showed reduced damage. We interpret this latter result as an example of per individual dilution of herbivory; with higher abundance of the preferred hosts, any given ramet received only moderate levels of damage. At the same initial densities of the *Trirhabda*, species growing in a mixed community of

hosts thus experienced different patterns of herbivory than would be expected given the damage these species receive when growing with close relatives. Our findings present a novel example of associational resistance and susceptibility in a community of co-occurring close relatives.

The variation in the levels of herbivory in relation to the neighbouring plants has been studied primarily with examples of associational resistance involving a highly palatable or attractive plant that receives reduced damage when surrounded by unpalatable plants (Agrawal et al. 2006, Jactel & Brockerhoff 2007, Barbosa et al. 2009). Alternatively, cases of associational susceptibility typically involve broadly polyphagous outbreaking species that consume their primary hosts and spill over onto other plant species (White & Whitham 2000). In communities of frequently co-occurring close relatives, such as in the Asteraceae-dominated old fields, damage by insect herbivores that can utilize multiple related hosts may strongly depend on the hierarchy of host use and the co-occurrence patterns of the host species. All of the focal species of goldenrods and asters frequently co-occur locally, even at the level of microsites, but monospecific or congeneric stands of goldenrods (esp. *So. altissima* and *So. rugosa*) are also common.

Life history traits of *Trirhabda* also suggest that local community structure may be an important determinant of damage, particularly during outbreaks. *Trirhabda* lays overwintering eggs on the soil and detritus, rather than directly on the host plants; the relatively mobile larvae have to search for suitable hosts; and the larvae have a propensity to switch plants even at low to moderate levels of damage (possibly due to an induced response), unless more food (esp. the primary host, *So. altissima*) is not available in the vicinity (Kessler et al., unpublished data). In sparse stands of goldenrods or on isolated genets, *Trirhabda* larvae tend to completely defoliate the preferred goldenrods before spilling over onto the less preferred asters (M.S. pers.

obs.). In contrast, dense monocultures of goldenrods may escape heavy defoliation through numeric dilution of individual herbivory, except in extreme outbreaks. These community-dependent patterns of herbivory may thus favour monospecific or congeneric stands of goldenrods (but see Long et al. 2003), and, at the same time, promote local coexistence of goldenrods and asters through the suppression of goldenrods by herbivory.

Herbivores have frequently been assumed, though less frequently shown, to play a major role in shaping of the structure of plant communities (Crawley 2009, but see Carson & Root 2000). However, few studies have explored how these top-down impacts of herbivory may be linked with bottom-up effects of plant community structure on the patterns of herbivory (Stein et al. 2010, Scherber et al. 2010). In our study, we have shown that patterns of herbivory by specialist *Trirhabda* larvae differ with community relatedness. Yet, in spite of two consecutive outbreaks, there has been little divergence in the structure of our focal communities compared to those that have been growing free of insect herbivores, irrespective of the community relatedness (Figure 4.4). We offer several interpretations of this result. First, perhaps a shift in the community structure under herbivory would only become apparent during a longer time scale. For instance, a longer-term study by Carson & Root (2000) observed a reduction in the dominance of *So. altissima* following several years of outbreaks by specialist Microrhopala vittata. Alternatively, the amount of herbivory by *Trirhabda*, in spite of two consecutive outbreaks, was not sufficient to produce a lasting impact on the community structure. However, our manipulated levels of herbivory were comparable to those observed in moderate *Trirhabda* outbreaks (M. S., pers. obs.), and under natural conditions most insect outbreaks tend to last for only 2-3 years (Carson & Root 2000). It is important to note that cumulative foliar herbivory by the end of the season did not differ between the community types for any of the species or overall.

Because most of the herbivory occurred during the *Trirhabda* outbreaks, and remained visible for the rest of the season, this result cannot be fully explained by late-season insect herbivory.

Instead, we suggest that these findings point to a remarkable level of tolerance against the effects of herbivory in this system. In particular, the large investment into below-ground biomass in goldenrods, which we were unable to measure, could be, in part, an adaptation to compensate for heavy herbivory by several outbreaking species in this system (Root & Cappuccino 1992; see also Uriarte 2000). The lack of differences in herbivory and its impacts between the mixtures and congeneric communities by the end of the season may then be attributed to regrowth following the outbreaks, suggesting that the resulting community structure may be shaped mostly by competitive interactions. The potential link between tolerance and competitive ability of a species remains an unexplored issue in a multi-species, community context. Previous work on outbreaking insects in goldenrods showed that the dominance of *So. altissima* in the community was reduced only following a multi-year outbreak of a narrow specialist, Microrhopala vittata (Chrysomelidae), in which both larvae and adults can reach outbreak densities, suggesting a high level of tolerance to more moderate levels of herbivory throughout the season (Cain et al. 1991, Carson & Root 2000). While our results also suggest that the goldenrod-aster community is highly resilient to the effects of insect herbivores, it is still possible that the feedback between plant community structure and herbivory may become more apparent over longer time scales (Carson & Root 2000).

In spite of this resilience, insect herbivory still played an important role in influencing local community structure in this system when the broader old-field plant community was considered. Specifically, we found that herbivory promoted the invasion of the experimental mesocosms by other, colonizing old-field species, both in

terms of their species richness as well as their overall abundance (Figure 4.5); these findings agree with previous studies in this system, that observed increased abundance or richness of old-field forbs following bouts of herbivory (Brown 1994, Carson & Root 2000). Although the levels of invasion were somewhat variable among the three community types, the overall interaction term between relatedness and herbivory was not significant, suggesting that the impact of herbivory on the success of the invading species was similar regardless of the community relatedness. This result is not surprising given that the three types of communities achieved similar levels of total productivity in the absence of herbivory, experienced similar proportional reductions in biomass in the herbivory treatment, and displayed little change in the community structure of the focal species due to herbivory.

The increased invasion of the communities under insect herbivory may have been facilitated through at least two mechanisms. First, other species may have found easier opportunities to colonize the mesocosms when the growth and competitive ability of the focal species, which constituted the majority of the community biomass, were suppressed by insect feeding damage. This explanation is particularly likely given that the most significant bout of herbivory in each year was the experimental outbreak of the specialist *Trirhabda* in the earlier part of the season, potentially opening a window of invasion opportunities for other, non-host species. It also suggests that herbivory by other insects on the colonizing species may have been relatively low, and competition with the focal species may have been a more important factor in the resistance against invasion, since the invaders did not increase in the insect-exclusion treatment. Alternatively, insect herbivory may have promoted increased richness of invaders by suppressing some of the most dominant colonizing species; the effects of herbivores on community structure via the release of competitively inferior species are well-documented (Crawley 1989). With either

mechanism, or their joint effects, insect herbivores may then contribute to the promotion and maintenance of local plant diversity in old-field communities.

Conclusion

The feedbacks between local plant community structure and herbivory are likely to be dynamic, as the populations of plants and herbivores vary in time and space (Root & Cappuccino 1992, Carson & Root 2000). Depending on the interplay of the bottom-up and top-down effects, herbivores and plants may thus exert directional versus stabilizing effects (in an ecological sense) on each other. For instance, an initial structure of the plant community may favour increased local herbivory, which in turn may lead to a shift in the composition of the plant community over time. However, the resulting change in the plant community structure may then cause a decline in the herbivore populations and their impact on the plant community, with other drivers of community structure (e.g. competition) becoming more important. The community may then begin shifting back towards the initial conditions, favouring the increase in herbivory and its top-down effects. While similar concepts have been discussed primarily in the context of population dynamics and regulation of plants and insects (Root and Cappuccino 1992), our study highlights the need to explicitly consider the spatial and temporal variation in the plant community structure, particularly in terms of the co-occurrence of related plant species, in order to enhance our understanding of community outcomes of plant-herbivore interactions and the maintenance of local diversity.

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SUMMARY

In my dissertation research, I examined whether plant species relatedness influenced the relative roles of plant competition and insect herbivory in structuring the local community of closely related, old-field Asteraceae. By focusing on closely related, co-occurring species (genus *Solidago*, *Symphyotrichum*, and *Euthamia*), the approach provided an explicit experimental test of the long-standing relatedness-competition hypothesis, which predicts that competition between close relatives should be stronger than competition among more distantly related taxa. My research also explored how plant community structure affects herbivory by a specialist insect herbivore, *Trirhabda virgata*, and, in turn, how the impacts of herbivory altered plant competitive dynamics and community structure.

The results from the pairwise competition study in a greenhouse did not support the relatedness-competition paradigm. I found that the outcome of competition, measured as the reduction of biomass of the target plant by its neighbour, was invariant with regard to the degree of relatedness between the two competing plants, across the six species. These findings are surprising, given the hierarchy of relatedness that included competing conspecifics from the same versus unrelated maternal lines, species competing with their close relatives (congeners), or with species from another, related genus. In other words, the intensity of intraspecific competition was comparable to that of interspecific competition regardless of the relatedness of the competing species.

While pairwise competition under greenhouse conditions may not be an accurate representation of the more diffuse competitive interactions in natural conditions, these findings were in agreement with the results from a three-year, field mesocosm experiment that manipulated community relatedness to evaluate the

intensity of competition using four complementary approaches. Competitive interactions when the focal species were growing in more closely related, congeneric communities compared to the more diverse, and more distantly related mixtures of the two genera, did not show differences in the expected direction. Specifically, the total community productivity of mixtures was not any higher than that expected from the productivities of the congeneric communities; the performance of each species did not vary between congeneric communities and mixtures in spite of the variation in their competitive abilities and dominance; and the phytometer species, although growing better in mixtures than expected, did not show stronger suppression when growing with its closest relatives. Collectively, these findings suggest that the focal species experienced similar competitive environments regardless of whether they were growing with their close relatives or in more diverse mixtures. However, the mixtures were less invaded by other, colonizing old-field species than predicted by the invasion of the congeneric communities. This result suggests that the increased resistance to invasion in the mixtures may have been conferred by a broader occupied niche space, and highlights the importance of competition in the continued assembly of the old-field community.

The patterns of host preference by outbreaking larvae of broad specialist *Trirhabda virgata* tended to be divided along the evolutionary relationships among the hosts: in spite of relatively weak differences in larval performance, all *Solidago* spp. were attacked more than the *Symphyotrichum* spp. This hierarchy was even stronger in adult *Trirhabda*, which showed a pronounced preference for the dominant, primary host *So. altissima*. Unlike in the more polyphagous larvae, adult performance was tightly linked to their preference hierarchy, in agreement with the predicted optimization of female fecundity given the insect life history traits. These patterns of host utilization by *Trirhabda* provided the basis for the prediction that the differences

in larval attack rates on the focal species may be contingent on the availability of the different hosts; at outbreak densities, the larvae can successfully feed on the less preferred hosts once the primary hosts are depleted. Therefore, at the scale of host plant patches, the goldenrod-aster communities may differ in the levels of *Trirhabda* feeding damage, and potentially be differentially impacted by herbivory.

These predictions were tested by contrasting how the communities of different relatedness responded to the two insect herbivory treatments in the mesocosm experiment: a two-year exclusion of insects with an insecticide spray versus two consecutive outbreaks by *Trirhabda* larvae. The host preference hierarchy of the larvae produced dramatically different levels of herbivory on the focal species depending on the community relatedness. Specifically, *Solidago* spp. showed associational susceptibility when growing mixed with the less preferred *Symphyotrichum* (which benefited from a type of associational resistance), as the larvae concentrated their feeding on the preferred hosts. In contrast, *Solidago* spp. suffered lower rates of defoliation in their congeneric communities, where their high densities resulted in per-capita dilution of damage. In the opposite pattern, *Symphyotrichum* spp. growing with their congeners experienced increased levels of herbivory compared to when growing in the mixtures, due to the absence of the preferred *Solidago* hosts. Therefore, the relatedness of the plant hosts in the local communities produced very different outcomes for specialist herbivory, driven by the preference hierarchy of this key outbreaking insect. However, contrary to predictions, differential herbivory between the relatedness treatments did not lead to a shift in the community structure, in spite of the two consecutive outbreaks. While insect herbivory reduced above-ground biomass of the community and of most of the focal species, by the end of the season the cumulative defoliation did not vary with community relatedness. This result appears to have been driven primarily by the high degree of

compensation and tolerance to herbivory in *Solidago* spp. On the other hand, insect herbivory promoted the invasion of mesocosms by other old-field species. These findings highlight the importance of initial community structure on the patterns of insect herbivory, and suggest that over longer time scales, these effects may lead to important feedbacks between herbivory and old-field community structure.

Collectively, the results presented in my dissertation argue for the usefulness of integrating the relatedness framework in studies of the factors that shape species interactions and community structure. Although I did not find support for the relatedness-competition paradigm in these co-occurring, closely related species, competition influenced broader structure of the plant communities. However, the relatedness of plants in the local community played an important role in the outcomes and mechanisms of resistance to an outbreaking insect herbivore. Driven by the host preference hierarchy of the herbivore, the patterns of herbivory were contingent on the community relatedness, implying the possibility that the structure of the plant community may both shape and be shaped by the top-down effects of herbivores. My research demonstrates the joint roles of competition and herbivory, and their possible feedbacks, in plant coexistence and maintenance of local diversity. On the other hand, it also suggests that the evolutionary relationships within a plant community are more important in the interactions of plants with other trophic levels, rather than in determining competitive outcomes among closely related plant taxa.